CMOS Contact Imagers for Spectrally-Multiplexed Fluorescence DNA Biosensing

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
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University of Toronto
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Abstract
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Within the realm of biosensing, DNA analysis has become an indispensable research tool in medicine, enabling the investigation of relationships among genes, proteins, and drugs. Conventional DNA microarray technology uses multiple lasers and complex optics, resulting in expensive and bulky systems which are not suitable for point-of-care medical diagnostics. The immobilization of DNA probes across the microarray substrate also results in substantial spatial variation. To mitigate the above shortcomings, this thesis presents a set of techniques developed for the CMOS image sensor for point-of-care spectrally-multiplexed fluorescent DNA sensing and other fluorescence biosensing applications.

First, a CMOS tunable-wavelength multi-color photogate (CPG) sensor is presented. The CPG exploits the absorption property of a polysilicon gate to form an optical filter, thus the sensor does not require an external color filter. A prototype has been fabricated in a standard 0.35μm digital CMOS technology and demonstrates intensity measurements of blue (450nm), green (520nm), and red (620nm) illumination.

Second, a wide dynamic range CMOS multi-color image sensor is presented. An analysis is performed for the wide dynamic-range, asynchronous self-reset with residue readout architecture where photon shot noise is taken into consideration. A prototype was fabricated in a standard 0.35μm CMOS process and is validated in color light sensing. The readout circuit achieves a measured dynamic range of 82dB with a peak SNR of 46.2dB.

Third, a low-power CMOS image sensor VLSI architecture for use with comparator-based ADCs is presented. By eliminating the in-pixel source follower, power consumption is reduced, compared to the conventional active pixel sensor. A 64×64 prototype
with a 10μm pixel pitch has been fabricated in a 0.35μm standard CMOS technology and validated experimentally.

Fourth, a spectrally-multiplexed fluorescence contact imaging microsystem for DNA analysis is presented. The microsystem has been quantitatively modeled and validated in the detection of marker gene sequences for spinal muscular atrophy disease and the *E. coli* bacteria. Spectral multiplexing enables the two DNA targets to be simultaneously detected with a measured detection limit of 240nM and 210nM of target concentration at a sample volume of 10μL for the green and red transduction channels, respectively.
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Acronyms

ADC analog-to-digital converter
APS active pixel sensor
BHQ black hole quencher
cADC coarse analog-to-digital converter
CCD charge-coupled device
CMOS complementary metal-oxide-semiconductor
CPG color photogate
CTIA capacitive transimpedance amplifier
DAC digital-to-analog converter
DNA deoxyribonucleic acid
DR dynamic range
ENOB effective number of bits
fADC fine analog-to-digital converter
FPGA field programmable gate array
FPN fixed pattern noise
FRET Förster resonance energy transfer
FWHM full width half maximum
gQD  green-emitting quantum dot
IR  infrared
LED  light-emitting diode
LFSR  linear feedback shift register
LSB  least significant bit
MOS  metal-oxide-semiconductor
MOSFET  metal-oxide-semiconductor field-effect transistor
MSB  most significant bit
ND  neutral density
PDMS  polydimethylsiloxane
PMT  photo multiplier tube
POC  point-of-care
QD  quantum dot
RGB  red-green-blue
RMS  root-mean-square
rQD  red-emitting quantum dot
SCA  split-comparator architecture
SMN  survival motor neuron
SNR  signal-to-noise ratio
VLSI  very large system integration
WDR  wide dynamic range
Chapter 1

Introduction

Analytical platforms are used in the life sciences for the observation, identification, and characterization of various biological systems. These platforms serve applications such as deoxyribonucleic acid (DNA) sequencing, immunoassays, and gene expression analyses for environmental, medical, forensics, and biohazard detection [1–3]. Biosensors are a subset of such platforms that can convey biological parameters in terms of electrical signals. Biosensors are utilized to measure the quantity of various biological analytes and are often required to be capable of specifically detecting multiple analytes simultaneously. A goal in biosensor research is to develop portable, hand-held devices for point-of-care (POC) use, for example, in a physician’s office, an ambulance, or at a hospital bedside that could provide time-critical information about a patient on the spot [4].

The current demand for high-throughput, point-of-care bio-recognition has introduced new technical challenges for biosensor design and implementation. Conventional biological tests are highly repetitive, labour intensive, and require a large sample volume [2,5]. The associated biochemical protocols often require hours or days to perform at a cost of hundreds of dollars per test. Instrumentation for performing such testing today is bulky, expensive, and requires considerable power consumption. Problems remain in detecting and quantifying low levels of biological compounds reliably, conveniently, safely, and quickly. Solving these problems will require the development of new techniques and sensors.
Figure 1.1: Schematic illustration of the deoxyribonucleic acid (DNA) double helix structure and associated binding behavior [7].

DNA analysis has proven to be invaluable in a wide range of applications [1, 6, 7]. These applications include drug development, gene expression profiling, functional genomics, mutational analysis, and pathogen detection. Therefore the detection of DNA is chosen to serve as a technology-driving motivation and a platform for validating the techniques developed in this research.

This introductory chapter is organized as follows. Section I describes DNA detection fundamentals. Section II details DNA detection techniques such as electrochemical detection, surface plasmon resonance, and laser-induced fluorescence. Section III and IV describes limits of existing optical DNA detection technologies and possible solutions, respectively. Section V details CMOS imager requirements for point-of-care fluorescence biosensing.

1.1 DNA Detection Fundamentals

The genetic blueprint of every living organism is defined by its genome and is contained in the sequence of nucleotide bases that make up the DNA [6]. Regions of DNA called genes are transcribed into ribonucleic acid (RNA) and are subsequently translated into
strings of amino acids. These amino acids are responsible for the formation of proteins, the major catalysts and structural components of the cellular world.

DNA exists as a double-stranded molecule in the cell nucleus and exhibits a three-dimensional structure known as a double helix. The two strands are held together by hydrogen bonds, as depicted in Fig. 1.1. There are four bases where Adenine, commonly abbreviated as ‘A’, always pairs with Thymine (‘T’) and Guanine (‘G’) always pairs with Cytosine (‘C’). This complementary base pairing allows the base pairs to be packed in the most energetically favourable arrangement. The double helix can be ‘denatured’ to form two single-stranded DNA (ssDNA) molecules. This is often accomplished through heating. Conversely, two complementary ssDNA molecules can form a double-stranded DNA molecule through the ‘renaturation’ process, commonly referred to as ‘hybridization’.

Sensors that function based on hybridization are called affinity-based sensors [3,8–10]. Affinity-based sensors detect the concentration of ‘target’ molecules, e.g., bacterial genes, in an analyte, e.g., food sample, based on their interactions with ‘probe’ molecules. The affinity of binding of a target strand to the probe molecule is governed by the degree of complementary between the two strand molecules. A target has a stronger affinity for its complement than it has for probes with a different sequence. When hybridization occurs, techniques exist to covert a hybridization event into a readable signal, for example, light intensity and charge distribution. Appropriate transducers are often used to convert such a change into an electronic signal for readout and analysis.

Sample preparation is often required prior to the hybridization process. Examples of sample preparation include target amplification to bring the target concentration to a sufficiently high level for detection and attachment of labels to generate a signal suitable with the detection platform. Fluorescent molecules are often used as labels. However, magnetic nanoparticles, gold nanoparticles, and enzymes have also been used.

Important measures of performance for affinity-based DNA biosensors include detection limit, selectivity, and dynamic range [9,10]. The detection limit is the lowest density of the target that can be reliably detected by the sensor, or in the case of a solution, the lowest target concentration that can be detected. In practice, a specific
signal-to-noise ratio (SNR) is often used to define the detection limit, e.g., an SNR of 3dB. The noise level can be obtained from the sensor response to a buffer (blank) solution.

Selectivity refers to the ability of a sensor to respond only to one type of DNA target sequence in a sample containing other (non-complementary) sequences. For nucleic acid detection, selectivity is often governed by the environment of hybridization.

The dynamic range of the sensor is the ratio of the highest to the lowest target concentrations that result in a predictable (linear or logarithmic) response from the sensor. The former parameter is often limited, in the chemistry, by the maximum amount of target molecules that can hybridize onto the probe layer (due to finite probe surface area) or, in the electronics domain, by the saturation level of the detector.

1.2 DNA Detection Techniques

Well-known DNA detection techniques include electrochemical detection [9,10], surface plasmon resonance [11], and laser-induced fluorescence [3,8].

1.2.1 Electrochemical Detection

In electrochemical DNA detection, a charge-transfer chemical reaction causes a change in the electrical properties of the system. Subcategories of electrochemical methods include cycle voltammetry, constant-potential amperometry, and impedance spectroscopy [9,10], which are often collectively referred to as electrochemical amperometry.
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Detection may be label-free or requires labeling. The labeled case is depicted in Fig. 1.2. In general, the system involves one or more electrodes (e.g., reference and working electrodes). Single-stranded DNA probes are first immobilized on the electrodes and then immersed in an electrolyte solution. Next, single-stranded DNA targets that have been labeled with an electroactive chemical are introduced to the electrolyte and allowed to interact with the probes. These labels are designed to transfer charge to the electrode when a potential is applied. Then, a potential is applied between the two electrodes and only labels attached to surface-hybridized targets are able to transfer charge to the electrode. A quantitative measure of the degree of hybridization or the target concentration is obtained by monitoring the reduction-oxidation current.

Although electrochemical amperometric sensing is well-suited for low-cost, portable applications, due to the inherent noise of performing electrochemistry in a solution, detection limit is often orders of magnitude higher in concentration than that of optical techniques [3,9,10].

1.2.2 Surface Plasmon Resonance

Surface plasmon refers to a collective oscillation of electrons in the conduction band of a thin conductive metal film. Surface plasmon resonance [11] refers to when the electric field of an incoming radiation (such as a laser source) is in resonance with the electric field of surface plasmons to stimulate excitation of surface plasmons. The hybridization of a target strand to a probe strand that is immobilized in close proximity to the thin metal film results in a change in the optical mass (refractive index and/or mass), which changes the resonance conditions for excitation of surface plasmons. This change is transduced in terms of a change in the incidence angle for maximal light absorption, which serves as an analytical signal. An advantage of using surface plasmon resonance for optical interrogation of nucleic acid detection is that it is a label-free technique. However, this technique is not easily applicable to an arrayed sensor implementation, which limits the overall sensor throughput.
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1.2.3 Laser-induced Fluorescence

Fluorescence-based transduction is a mature technique and finds a multitude of applications in the life sciences. In particular, laser-induced fluorescence is a prominent sensory method for lab-on-a-chip devices [12]. For many analytes, it provides the highest sensitivity and selectivity [13]. As a result, fluorescence is the most widely used, with applications ranging from cancer diagnostics [1,14] to genetic research [8,13].

Figure 1.3: Key spectra of the commonly utilized Cyanine3 (Cy3) fluorescent molecule [15].

In the standard form of laser-induced fluorescence [2], a fluorescent molecule, also known as a fluorescent label or a fluorophore, is attached to each of the target molecules through a process called labeling. The fluorophore, upon absorbing photons at one wavelength, emits photons at a longer wavelength. Fluorophores such as Cy3, Cy5, and fluorescein are commonly used as fluorescent molecules and usually emit light with the wavelength in the 500nm to 700nm range. Fig. 1.3 adopted from [15], depicts the absorption and emission spectra of the Cy3 fluorophore. Multiple fluorophores, e.g., green and red labels, are sometimes used for color multiplexing, i.e., to screen for multiple targets.

Upon excitation, light is given off if hybridization occurs, i.e., the probe found its matching target. Fluorophores that are not bound to any probe do not produce an optical signal. Depending on the specific assay method, fluorophores are either
Figure 1.4: Hybridization-based DNA detection on a microarray. Samples are labeled with fluorescent molecules.

chemically inactive (do not produce light) or are removed by a washing step. Typically the light is collected with a photodetector after passing through an optical filter that rejects any stray excitation light.

Due to the superior sensitivity, linearity, and suitability for spectrally multiplexed optical detection, fluorescence is chosen to be the transduction method for this work.

### 1.3 Limits of Existing Optical DNA Detection Technologies

Fluorescence-based microarrays have arguably become the standard optical DNA detection technology [3]. Microarrays enable highly-multiplexed and parallel detection, depicted in Fig. 1.4 adopted from [16]. Typically, single-stranded DNA (ssDNA) probe molecules are arranged in a regular pattern on a passive substrate, such as a glass slide. Probes are then allowed to hybridize with complementary, fluorophore-labeled ssDNA target molecules. After that, non-hybridized targets, i.e., those not sought after, are removed from the array through a washing step. The hybridized targets are then detected by an instrument.

Although the DNA microarray is a widely adopted technology, it is not without its drawbacks. In terms of the assay, although the spatial registration of probes in
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Figure 1.5: An image of a cluster of spots on a typical microarray illustrating high variability.

DNA microarrays exhibits unprecedented parallelism, it suffers from disadvantages such as additional processing required to print spots on a surface and the associated spatial variation in terms of the quality of the probe immobilization across discrete spots [2][17]. Immobilizing probe molecules on a surface consistently across the array is a challenge, as shown in Fig. 1.5 where high variability between spots can be seen. Also, microarray technology employs a hybridization chemistry which requires the washing away of samples after introducing them to the probes. This renders real-time sensing difficult, often prohibitive. Therefore only the outcome rather than the dynamics of the biological experiment can be observed. In addition, microarray manufacturing typically involves time-consuming processes and a laboratory environment.

In terms of the instrumentation, the multiple lasers and optical detectors employed in the microarray scanners and the widely-used fluorescent microscope render them bulky, which limits portability for point-of-care applications [8]. Fig. 1.6 depicts the light signal paths of a typical fluorescent microscope. Excitation is typically provided by a laser source, which passes through an excitation filter to remove stray outputs that overlap with the emission wavelengths. Then, the dichroic mirror reflects and directs the excitation to the sample. The fluorophores in the sample absorbs the excitation light and emits light at a longer wavelength, which is passed through the dichroic mirror. The emitted light is then directed through an emission filter (for choosing the exact wavelength in a multi-wavelength emission setup), a focusing lens, an aperture (to control the output intensity to prevent detector saturation), and finally reaches the detector, typically a photomultiplier tube (PMT) or a cooled charge-coupled device.
Figure 1.6: Schematic of a microscopy setup commonly utilized in fluorescence sensing experiments.

(CCD). In addition to limited portability, the high instrumentation cost associated with microarray platforms can be prohibitive for many applications.

1.4 Possible Solutions to Existing Technological Limitations

Reduction in platform complexity, form-factor, and cost can be achieved using alternative detection methods and technologies other than the traditional laser-induced fluorescence based DNA microarrays. The following approaches are used to meet the aforementioned challenges.

1.4.1 Spectral Multiplexing

As discussed previously, conventional array-based fluorescent sensing technologies, such as DNA microarrays, rely on spatial registration of probes to achieve multiplexed detection of target analytes. This requires additional steps for the preparation of surface
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chemistry, which is prone to spatial variation. One of the advantages of fluorescence-based sensing is its suitability for spectral multiplexing, which eliminates the need for spatial registration and the associated spatial variation. Target analytes can be labeled with different fluorophores that can be distinguished by their emission wavelengths. By measuring the emission intensity at each of these wavelengths, different targets, such as nucleic acid targets, can be simultaneously quantified [18].

1.4.2 Microsystem Integration

The need for portability can be met with microsystem integration. Integrated circuit based DNA detection platforms (e.g., CMOS biosensors) have a great potential for point-of-care diagnostic applications because they can be integrated with other technologies to construct compact, self-contained sensing platforms. For example, it is envisioned that biochemical sample preparation could be performed using microfluidic channels with integrated pumps and valves; on-chip solid-state transducers (e.g., CMOS photodiodes) could be used to detect specific DNA sequences in an analyte; and microelectronic integrated circuits could amplify and condition the transducer output signal, convert this information to a digital format, process it in order to extract relevant biochemical data, and then transmit or display these data externally [5,7].

In addition, innovation in imaging techniques such as contact imaging [8,19,20] can be employed to reduce the size of the conventional bulky and expensive optical detection instruments. Unlike the conventional fluorescent microscope, in contact imaging, the object to be imaged is placed on or in close proximity to the focal plane.

1.4.3 Image Sensing with CMOS

The choices of the photodetector for fluorescence imaging systems have conventionally been the PMT and the CCD. PMTs are amongst the most sensitive photodetectors, but are bulky, expensive and require high operational voltage making them unattractive to be integrated into a portable system. The throughput of PMT-based detection systems is relatively low due to the lack of parallelism. In contrast, CCDs can be implemented into an array, but do not allow for on-chip integration of peripheral cir-
1.5 Spectroscopy versus Colorimetry

Spectral-multiplexing, which is the technique employed for DNA detection in this thesis as explained in Sec. 1.4.1, is a spectroscopic technique requiring coarse wavelength resolution. Since spectroscopy can be confused with the related field of colorimetry, as employed in color imaging, the difference between the two techniques are detailed in this section.

In spectroscopy, a spectrometer measures spectral data – the amount of optical energy from an object at several intervals along the spectrum, as depicted in Fig. 1.7(a). These values are typically represented as a spectral curve. The optical energy can be reflected from, transmitted through, or emitted by an object. Spectral data is illuminant-independent, which means that lighting changes and the uniqueness of each human viewer have no effect on the data.

On the other hand, in colorimetry, a color camera or colorimeter measures and computes the light intensity reflected from or transmitted through an object. Colorimetry quantifies and describes light with respect to physical human color perception, as
depicted in Fig. 1.7(b). It reduces the spectra to the physical correlates of color perception, most often in the form of tristimulus values as defined in the standard CIE 1931 XYZ color space, put forward by the International Commission on Illumination (CIE) [22]. This standard assumes color reproduction by combining emitted lights (e.g., from a liquid crystal display) to create the sensation of a range of colors based on an additive color system. Typically, the primary colors used are red, green, and blue, but other colors are also used in specific applications to enlarge the color gamut.

Noteworthy properties of spectroscopy and colorimetry are highlighted below [23]:

In colorimetry, the objective is to reproduce color to look natural to the human: Primary colors are not a fundamental property of light but are related to the physiological response of the eye to light. Fundamentally, light is a continuous spectrum of the wavelengths that can be detected by the human eye. However, the human eye normally contains only three types of color receptors, called cones. Each color receptor responds to different ranges of the color spectrum. Humans and other species with three such types of color receptors are known as trichromats. These species respond to the light stimulus via a three-dimensional sensation, hence three primary colors are often used.

Spectroscopy and colorimetry have different spectral coverage requirements: Since the purpose of colorimetry is to describe color as perceived by the human, colorimetry requires spectral sensing of only the visible wavelengths from 380nm to 720nm, as depicted in Fig. 1.8(b). On the other hand, it is not uncommon that spectroscopy also
include the ultra-violet (UV) and infrared (IR) wavelengths. However, the spectra of
interest in spectroscopy can be discontinuous, as depicted in Fig. L8(a). For example,
in fluorescence imaging, the emission wavelengths of fluorophores are known a priori,
therefore only the wavelengths of interest are required to be sensed.

Wavelengths of interest in spectroscopy are not necessarily most efficiently captured
by colorimetric sensors: The human photoreceptors have sensitivity peaks at short
(430nm), middle (535nm), and long (570nm) wavelengths. Therefore, colorimetric
systems use these wavelengths as primary colors. As an example, in contrast, the Cy5
and Alexa647 fluorophores have peak emissions at 575nm and 647nm, respectively.
Thus, color sensors that are tailored for human vision is not necessarily most suitable
for spectroscopy.

Colorimetry requires spectral overlaps in its primary-color filters but spectroscopy
requires spectrally non-overlapping filters: In colorimetry, colors that fall in between
primary colors are sensed by combining the reading of two or more primary colors.
For example, the color yellow is inferred from a signal registered by both the green
and red filters. Therefore, it is desirable to have a set of primary color filters with
a relatively wide spectral coverage to the point that they spectrally overlap. On the
other hand, spectroscopy usually involves the traversing of a filtering mechanism, e.g.,
grating, across the spectrum. Since the objective is to sense the optical energy at each
bin of wavelengths, it is ideal to have as narrow a response as possible from this filter.

Full spectral data can be converted to colorimetric data but not vice versa: A full
set of spectral data capture the power distributions of the light sources, whereas col-
orimetry only captures color perception for the human. As an example, consider two
light sources made up of different mixtures of various wavelengths. These light sources
may have the same apparent color to a human observer when they produce the same
tristimulus values, but the spectral power distributions of the sources may be different.
1.6 CMOS Imager Requirements for Point-of-Care Fluorescence Biosensing

The transition of fluorescence biosensory imaging instruments to a microsystem form factor gives rise to the need to integrate multiple key functionalities into the sensor [8–10]. In this work, the following needs are identified and selected to be addressed. First, there is a need to differentiate among the multiple emission wavelengths from the multiple colors of fluorophores. Spectroscopic sensing can be integrated onto the imager chip to simplify emission light filtering, thus simplifying the overall system. Second, since chemical concentrations can vary by orders of magnitude [3, 24], sensors need to accommodate a wide input dynamic range. Third, since point-of-care instruments are often required to be portable, low power consumption is necessary. Fourth, the CMOS sensor must be readily integrated with the rest of the microsystem to deliver high overall system performance to meet the requirements of real-world applications. Being the core component, the CMOS integrated circuit performance often dictates the overall system performance. Therefore, both the functionality and performance of the CMOS sensor is of critical importance.

1.7 Thesis Organization

This thesis presents a set of techniques developed for CMOS imagers employed in fluorescence sensing applications to meet the needs listed in the previous section. This chapter serves as the motivation and provides the background for the thesis. The remainder of this thesis presents four key contributions addressing the four needs organized into their respective chapters with each one containing quantitative analysis, original design, and experimental validation in silicon.

Chapter 2 presents a CMOS photogate sensor. The sensor spectrally differentiates among multiple emission bands, replacing the functionality of a bank of emission filters in a conventional fluorescence detection system. This integration of spectral differentiation capability onto the imager eliminates the need to mechanically swap band-pass optical filters in a multi-color fluorescence imaging microsystem, a procedure that is
Chapter 1. Introduction

difficult, if not impossible to perform under such space constraints.

Chapter 3 presents a dynamic range extension technique for CMOS imagers. An analysis is presented for the *wide-dynamic-range asynchronous self-reset with residue readout* architecture where photon shot noise is taken into consideration. This technique optimizes circuit area and power consumption as the imager analog-to-digital converter (ADC) adjusts its intrinsic noise according to the input-dependent shot-noise level in the system.

Chapter 4 presents a low-power CMOS imager VLSI architecture for comparator-based ADCs. A single column-parallel comparator is split among all pixels in the column. This allows the pixel to have a compact three-transistor circuit implementation, which maintains the same transistor count as the high-density conventional active pixel sensor (APS). By eliminating the in-pixel source follower, power consumption is reduced, compared to the conventional APS.

Chapter 5 presents a spectrally-multiplexed fluorescence contact imaging microsystem for DNA analysis. The multi-color imaging capability of the microsystem in analyzing DNA targets has been validated in the detection of marker gene sequences for the spinal muscular atrophy disease and the *E. coli* pathogen. Spectral multiplexing enables the two DNA targets to be detected simultaneously, without spatial registering.

Chapter 6 summarizes the complete research and suggests future work.
Chapter 2

CMOS Tunable-Wavelength Multi-Color Photogate Sensor

2.1 Introduction and Prior Art

The differentiation among fluorescent emission wavelengths is essential to multi-color fluorescent imaging. In this chapter, a CMOS color photogate sensor is presented that performs this task of wavelength differentiation.

Conventionally, differentiation between fluorescent emission wavelengths has been performed by using a set of optical bandpass filters to select different parts of the emission spectrum [27], as shown in Fig. 2.1(a) for the case of a p-n-junction diode photodetector. The optics involved is bulky and expensive. To circumvent this problem, filterless spectral sensing methods have also been investigated. Methods based on diffraction grating (the splitting of light) [28] and Fabry-Perot etalon (tuned resonance cavity) [29] generally offer high spectral resolution, but require micromachining and post-processing such as wafer polishing and wafer bonding. Eliminating the need for sophisticated optics and post-processing is the ultimate remedy to high design complexity and fabrication cost.

Techniques that solely rely on integrated circuit process technology have been developed, most notably buried junction technology [25], on which the Foveon sensor is based, as shown in Fig. 2.1(b). Since light absorption in a semiconductor varies across
Figure 2.1: Filterless integrated circuit spectral sensing approaches. (a) Buried triple $p$-$n$-junction embedding diodes at three fixed depths [25], (b) photo sensing region depth modulation enabling light collection at multiple electronically-tunable depths in custom CMOS [26], and (c) proposed standard-CMOS tunable-wavelength multi-color photogate consisting two sensing regions, one of which is tunable by a voltage bias and covered by a poly-Si gate.

wavelengths in such a way that light of a longer wavelength can penetrate deeper, a photocurrent measured at a deeper depth consists of stronger longer-wavelength components. By sensing at several depths, color information can be inferred. Although the buried junction approach achieves high spatial density and is suitable for photographic applications requiring only three colors (e.g., blue, green, and red), there is a limit to the number of diodes that can be implemented, for example three for a dual-well process. This renders it unsuitable for applications that require spectroscopic sensing of more than three discrete bands of wavelengths. To overcome this limitation, a spectrally-sensitive photodiode has been developed [30], as shown in Fig. 2.1(c). A biased poly-silicon gate modulates the photo sensing region depth to effectively achieve an equivalent of many buried $p$-$n$-junctions. However, the reliance on the vertical dimensions of the CMOS process technology limits the scalability of the device dimensions. The most recently reported prototype is fabricated in a $5\mu$m custom process [30].

Compared to PMT and CCD, the CMOS technology has the advantages of low cost, high integration density, and signal processing versatility. Numerous recent designs based on the CMOS $p$-$n$-junction photodiode have been reported including a time-resolved fluorescent imager [20] and a lab-on-chip fluorometer [21]. The monochromatic
photogate typically used in a CCD has been demonstrated in CMOS [31]. However, the exploitation of the polysilicon gate for color sensing has largely been unexplored.

In this paper, we present a single-pixel tunable-wavelength multi-color photogate (CPG) sensor implemented in a standard digital 0.35μm CMOS technology, validated in spectrally-multiplexed fluorescence contact sensing. Sensing of a small set of well-separated wavelengths (e.g., >50nm apart) is based on tuning the spectral response of the CPG structure, as shown in Fig. 2.1(c). The structure consists of two sensing regions, one of which can be modulated by a voltage bias to modify the overall CPG spectral response. The CPG has a structural resemblance to the conventional CMOS monochromatic photogate [31] but it employs the polysilicon gate as an optical filter, thus requiring no external optical color (i.e., band-pass) filters. The CPG is designed to sense light intensity of multiple wavelengths which are known a priori, hence, it is suitable for coarse color differentiation in multi-color fluorescence applications as the fluorescence emission colors are known before detection. The overall integrated sensor consists of the CMOS tunable color photogate, an on-chip analog-to-digital converter (ADC), and a software algorithm to reconstruct the input light intensities at specific wavelengths. The CPG has been validated in quantum dot fluorescence measurements where only one long-pass optical filter to attenuate the excitation light (but not to distinguish among emission light colors) is required.

The rest of the chapter is organized as follows. Section II discusses the conceptual model of the CPG sensor. Section III details the VLSI implementation of the sensor. Section IV details the principle of operation. Sections V and VI report experimental results in light-emitting diode (LED) light measurements and QD fluorescence measurements, respectively. Section VII highlights key observations.

2.2 Conceptual Model

The tunable-wavelength multi-color photogate sensor measures the intensities of a small set of well-separated wavelengths (e.g., >50nm apart). The principle of operation is first illustrated by an example and is subsequently formulated analytically.
2.2.1 Concept of Tunable Spectral Responsivity

Unlike the buried junction approach [25] that employs multiple discrete photodiodes, the CPG creates the equivalent of multiple photodetectors by tuning the spectral responsivity of a single detector through modulating a control parameter, $p$, which can be implemented as a bias voltage.

To illustrate, Fig. 2.2(a) presents a device whose response to the control parameter, $p$, is ideal, for two colors. For example, to sense the green color, a measurement can be performed by setting the control parameter $p$ to P1. Similarly, to sense the red color, the control parameter $p$ is set to P2.

In practice, a device response may resemble that depicted in Fig. 2.2(b), where the device is sensitive to more than one color for any value of $p$. Hence, the device output current contains a mixture of color components. In this case, one method to determine the intensity at each wavelength is by analyzing multiple measurements, each using a unique value of $p$, then solving for the input intensity for each color. For example, to sense the intensities at the green and red wavelengths, two measurements are required with the control parameter set to values P1 and P2. As illustrated in Fig. 2.2(b), each measurement is a linear combination of scaled color intensities. Also, the change in the device response with respect to $p$ can be small, which is the key reason why in practice the device is restricted to the sensing of several well-separated wavelengths.
Chapter 2. CMOS Tunable-Wavelength Photogate

There are three types of variables involved: the detector responsivity to a particular wavelength, the measured photocurrent, and the input light intensity. A model of detector responsivity can be generated a priori, for example by measurement with known inputs. The input light intensity can then be calculated based on the set of measured photocurrents and the stored model.

2.2.2 Analytical Formulation

The above concept can be formulated analytically as follows. When the CPG is illuminated, the absorption of light is described by the Beer-Lambert law [32]. The absorbed photons generate electron-hole pairs, giving rise to a photocurrent for a single wavelength input that is given by

\[ I = \frac{qS\lambda}{hc} (1 - e^{-\alpha(\lambda)D(p)}) A'(\lambda) \phi \]  \hspace{1cm} (2.1)

where \( \phi \) is the radiation intensity, \( q \) is the elementary charge, \( S \) is the area of the detector, \( \lambda \) is the wavelength, \( h \) is Planck’s constant, \( c \) is the speed of light in vacuum, \( \alpha \) is the absorption coefficient, \( D \) is the effective depth of the sensing region, and \( A'(\lambda) \) is the absorption of a polysilicon gate structure. The absorption coefficient \( \alpha \) is a function of \( \lambda \). The aforementioned control parameter \( p \) determines the value of \( D \). For a given detector size, equation (2.1) can be rewritten as

\[ I = k(p, \lambda) \phi \]  \hspace{1cm} (2.2)

where \( k(p, \lambda) \) is the responsivity of the CPG and can be obtained empirically.

When light rays of multiple wavelengths are incident simultaneously, the photocurrent can be expressed as a linear combination of the CPG response at each wavelength. To determine the light intensities at each wavelength, multiple measurements are required. For example, for a two-wavelength input, the photocurrents \( I_1 \) and \( I_2 \) measured by the photodetector can be related to the input intensities \( \phi_1 \) and \( \phi_2 \) (at \( \lambda_1 \) and \( \lambda_2 \), respectively) by

\[ I_1 = k_{11}\phi_1 + k_{12}\phi_2 \]  \hspace{1cm} (2.3)

\[ I_2 = k_{21}\phi_1 + k_{22}\phi_2 \]  \hspace{1cm} (2.4)
where the $k$-coefficients are such that $k_{ij}$ is the detector responsivity under $i$-th control parameter to the $j$-th wavelength for $i=1,2$, and $j=1,2$. The input intensities $\phi_1$ and $\phi_2$ can be obtained by solving the system of equations, provided that the detectors have unique spectral responses (i.e., equations (2.3) and (2.4) are linearly independent). This model can be extended to a finite set of $N$ wavelengths. To determine the incident light intensity of an input spectrum to a resolution of $N$ distinct wavelengths, $N$ measurements are required, each with a different control parameter. Equations (2.3) and (2.4) thus extend to the $N$-variable system of equations

$$
\begin{bmatrix}
I_1 \\
I_2 \\
\vdots \\
I_N
\end{bmatrix} =
\begin{bmatrix}
k_{11} & k_{12} & \cdots & k_{1N} \\
k_{21} & k_{22} & \cdots & k_{2N} \\
\vdots & \vdots & \ddots & \vdots \\
k_{N1} & k_{N2} & \cdots & k_{NN}
\end{bmatrix}
\begin{bmatrix}
\phi_1 \\
\phi_2 \\
\vdots \\
\phi_N
\end{bmatrix}
$$

(2.5)

To empirically construct an $N \times N$ $k$-matrix model depicted in the system of equations (2.5), each $k$-coefficient is obtained by measuring the CPG photocurrent using a known illumination and the corresponding control parameter. For example, $k_{11}$ is obtained by inputting $\phi_1$ (a known intensity at wavelength 1) and measuring the CPG photocurrent under the control parameter $p_1$. Analogously, $k_{12}$ is obtained from $\phi_2$ and $p_1$, and $k_{21}$ is obtained from $\phi_1$ and $p_2$. This process is repeated $N \times N$ times to build the entire $k$-matrix. This computation is only performed once so this computation load is minimal. The model of equations (2.5) is then used to solve for the $N$ unknown light intensities $\phi$ based on $N$ measured currents $I$.

Provided that the wavelengths are well-separated, this method offers the flexibility to tune to an arbitrary set of wavelengths within the sensitivity range of the silicon photodiode. However, one limitation of this approach is that it requires the complete set of sensor input wavelengths be known a priori so that the appropriate $k$-coefficient model can be developed. As counterexamples, the sensor would report incorrect intensities if the input wavelengths differ from that of the model used in reconstruction, or if three wavelengths are present at the input but only a two-wavelength model is used.
Chapter 2. CMOS Tunable-Wavelength Photogate

2.3 Principle of Operation

2.3.1 Qualitative Analysis

In polysilicon, light is absorbed exponentially as a function of penetration depth \( \alpha \). Optical transmittance \( T \), the portion of light that passes through a layer of polysilicon with thickness \( l \) can be approximated as

\[
T_{\text{gate}} = e^{-\alpha(\lambda)l}
\]

where \( \alpha(\lambda) \) is the wavelength-dependent absorption coefficient, with values 3.56, 1.35, and 0.45\( \mu m^{-1} \) for the wavelengths of 450nm (blue), 520nm (green), and 620nm (red), respectively [32]. Fig. 2.3 depicts the optical transmittance of a polysilicon layer calculated based on the aforementioned absorption coefficients. For example, in the 0.35\( \mu m \) CMOS process, the thickness of the polysilicon gate is approximately 300nm [33], leading to an approximate transmittance of 35% for blue light (450nm), 70% for green light (520nm), and 85% for red light (620nm). This property of the polysilicon MOS gate is utilized in the color photogate design. It is worth noting that the gate is a well-fabricated structure in the CMOS process, with an intra-die thickness variation on the order of 3% [34]. The resulting variation of the optical transmittance can be observed in Fig. 2.3.

Figure 2.3: Simulated wavelength-dependent optical transmittance of polysilicon.
Chapter 2. CMOS Tunable-Wavelength Photogate

The color photogate (CPG) is schematically depicted in Fig. 2.4(a), structurally resembling the conventional surface-channel monochromatic CMOS photogate [31]. The core sensing region of the CPG is the large area covered by the polysilicon gate. A small $p^+$-diffusion, referred to as the edge region, forms the device output. A $n^+$-diffusion fabricated in an $n$-type body forms the ohmic bias contact. The $p^+$-output diffusion is set by the readout circuit to a voltage lower than the $n$-body voltage to maintain a reverse biased $p$-$n$-junction.

The gate performs two key functions for color sensing. First, it functions as an optical filter to provide wavelength-dependent absorption as described above. Second, it is a terminal for the induction of an electric field to modulate the extent of photo-generated carrier collection in the core region, the area under the gate. The gate-to-body biasing voltage $V_{GB}$ acts as the control parameter, $p$. When $V_{GB}$ is applied such that no depletion region is formed under the gate, as depicted in Fig. 2.4(a) cases A and B, photo detection only takes place near the $p^+/n$-body depletion region. As depicted in Fig. 2.4(a) cases C through E, when another $V_{GB}$ is applied to form a depletion region at the CPG core, it also participates in photo detection. But the light experiences wavelength-dependent absorption as it travels through the gate. Since the gate provides greater attenuation at shorter wavelengths, the core region provides additional long-wavelength (e.g., red) responsivity to the CPG. Since the edge and core of the CPG have different spectral properties, when different gate voltages are applied, an equivalent of multiple detectors with unique spectral responses is created, e.g., for two colors, equations (2.3) and (2.4) are implemented by a single device.

To understand the formation of the depletion region at the device core, the respective energy profiles of the CPG are depicted in Fig. 2.4(b). Depicted energy levels are the gate Fermi level $E_{Fm}$, semiconductor Fermi level $E_{Fs}$, intrinsic semiconductor Fermi level $E_i$, substrate conduction band $E_C$, and valence band $E_V$. As $V_{GB}$ changes, the CPG transits through modes of operations, analogous to a metal-oxide-semiconductor (MOS) capacitor. This mode change leads to a change in carrier collection efficiency, which when functioning with the wavelength-dependent absorption of the gate, leads to a change in device spectral properties.

Depicted in Fig. 2.4 case A, at a high gate-to-body bias, $V_{GB} >> 0$, the electric field
Figure 2.4: CPG modes of operation are illustrated as various cases: (A) accumulation, (B) flat band, (C) zero bias, (D) depletion, with illustration of photo-generated carrier flow, and (E) inversion. (a) cross-section diagram illustrating the location of carriers, and (b) energy band diagram illustrating the modes of operation of the CPG with a polysilicon gate and a $n$-doped body. Depicted are the gate Fermi level $E_{Fm}$, substrate conduction band $E_C$, semiconductor Fermi level $E_{Fs}$, intrinsic semiconductor Fermi level $E_i$, and valence band $E_V$. $V_{GB}$ is the gate-to-body voltage.
from the gate attracts electrons to the surface of the $n$-body. This is the accumulation mode. The high density of electrons in the surface layer of the $n$-body is exactly matched by the high density of holes at the gate, induced by the positive gate voltage applied. The appearance of extra electrons in the surface region of the substrate means that the Fermi level, $E_{Fs}$, in the surface region is close to the conduction band, $E_{C}$. The energy levels ($E_{C}$, $E_{i}$, and $E_{V}$) are, therefore, bent downwards going from the silicon substrate toward the gate. In this mode, the CPG core region is inactive. Photo detection only takes place in the $p^{+}/n$-body depletion region at the edge region of the device, hence producing a small photocurrent.

Depicted in Fig. 2.4 case B, as $V_{GB}$ decreases, the CPG enters the flat band mode, where the electrons in the $n$-body are compensated by the positive donor ions and the minority holes. Due to the work function difference between the gate and the substrate, the Fermi levels of the gate, $E_{Fm}$, and the substrate, $E_{Fs}$, are different. This difference is related to the flat band voltage $V_{FB}$. Therefore, the flat band condition generally does not occur at zero bias [32]. The only depletion region is at the edge $p^{+}/n$-body junction. As $V_{GB}$ reduces, passing the flat band biasing point, the CPG core begins to develop favorable potential for the collection of photo-generated carriers, which leads to a photocurrent contribution from the core.

Depicted in Fig. 2.4 case C, as $V_{GB}$ reaches zero, the CPG enters the zero bias mode. The Fermi level is constant throughout the system due to thermal equilibrium. There is a potential difference between the gate and the $n$-body at zero bias. This is analogous to the built-in voltage in $p$-$n$-junctions [32]. In this mode, a shallow depletion region is formed in the core region since the biasing condition deviates from that required for the flat band condition.

Depicted in Fig. 2.4 case D, when a small voltage $V_{GB} < 0$ is applied, the electric field produced repels the electrons from the surface creating a depletion layer at the surface of the silicon substrate. The CPG core is thoroughly depleted at this surface when the $n$-body intrinsic Fermi level $E_{i}$ equals $E_{Fs}$ there. The thoroughly depleted CPG has a well-developed depletion region and associated electric field to collect photo-generated carriers, therefore the photocurrent reaches a high level.

Depicted in Fig. 2.4 case E, when $V_{GB} << 0$, the CPG enters the strong inversion
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mode. As the energy difference between the valence-band \( E_V \) and the substrate Fermi level \( E_{Fs} \) is reduced, holes begin to appear at the surface of the substrate. However, these additional holes do not participate in photo-sensing, which results in a relatively high photocurrent, approximately constant across both the depletion and strong inversion modes.

The CPG in both the depletion mode and inversion mode has a well-developed depletion region to collect carriers as depicted in Fig. 2.4 cases D and E. Fig. 2.4 case D depicts the flow of photo-induced charge carriers within the CPG. Due to the photo-electric effect, when light of sufficient energy breaks a bond, creating an electron-hole pair, carriers travel via several different mechanisms as follows: (1) minority carrier holes in \( n \)-body travel to depletion region via diffusion formed by a carrier concentration gradient; this gradient is formed by the fact that the depletion region is deprived of carriers; (2) holes in the depletion region travel to \( p^+ \)-output diffusion via drift induced by the electric field resulting from the space charges of the \( p^+/n \)-body junction, and (3) majority carrier holes in \( p^+ \)-output diffusion travel to the output electrode by drift due to the (low) potential at the electrode. Photo-generated electrons drift to the \( n^+ \) ohmic contact due to its (high) applied potential and are discharged, i.e., not collected as a part of the photocurrent.

2.3.2 Quantitative Analysis

Given that the electric field in the depletion layer immediately separates the photo-generated electrons and holes, an expression can be written for the photocurrent based on the external generation rate \( G_{ext} \). The external generation rate, in contrast to generation due to thermal mechanisms, is the number of electron-hole pairs generated in a unit of the depletion-layer volume per second. The photocurrent of a \( p-n \)-junction photodiode is given by \[32\]

\[
I_{ph} = qG_{ext}A_jD_j
\]

(2.7)

where \( q \) is the elementary charge, \( A_j \) is the \( p-n \)-junction area (where the depletion layer is formed), and \( D_j \) is the depletion region depth. Since uniform carrier generation in the sensing volume and complete carrier collection are assumed, \( D_j \) is an approximated
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value. The external generation rate $G_{\text{ext}}$ can be related to the incoming optical input as

$$G_{\text{ext}} = \frac{P_{d,\text{in}} R(\lambda)}{q}$$

(2.8)

where $P_{d,\text{in}}$ is the input optical power density, in the units of $W/\mu m^3$, and $R(\lambda)$ is the responsivity of the detector, in $A/W$, which is wavelength-dependent.

Since the CPG has both the core region and the edge region, its photocurrent can be modeled as the sum of photocurrents in these regions. The photocurrent of the core region, $I_{\text{ph,core}}$, which is $V_{GB}$-dependent, is given by

$$I_{\text{ph,core}}(V_{GB}) = P_{d,\text{in}} T_{\text{gate}} R_{\text{core}}(\lambda) A_{\text{core}} D_{\text{core}}(V_{GB})$$

(2.9)

where $T_{\text{gate}}$ is the transmittance of the polysilicon gate, $R_{\text{core}}(\lambda)$ is the responsivity of the core region, $A_{\text{core}}$ is the core area, and $D_{\text{core}}(V_{GB})$ is the core sensing depth, which is dependent on $V_{GB}$. $T_{\text{gate}}$ is utilized to model the attenuation of the input light by the polysilicon gate.

Analogously, the photocurrent of the edge region, $I_{\text{ph,edge}}$ is given by

$$I_{\text{ph,edge}} = P_{d,\text{in}} R_{\text{edge}}(\lambda) A_{\text{edge}} D_{\text{edge}}$$

(2.10)

where $R_{\text{edge}}(\lambda)$, $A_{\text{edge}}$, and $D_{\text{edge}}$ are the responsivity, area, and depth of the edge sensing region, respectively.

To gain insight into $D_{\text{core}}(V_{GB})$, next, the relationship between depletion depth and $V_{GB}$ is formulated based on three regimes. For $V_{GB} > V_{FB}$, where $V_{FB}$ is the flat band voltage, there is no depletion in the CPG core

$$D_{\text{core}} = 0 \quad (V_{GB} > V_{FB})$$

(2.11)

For $V_{\text{INV}} < V_{GB} < V_{FB}$, where $V_{\text{INV}}$ is the voltage that triggers the onset of inversion, the depletion depth grows with decreasing $V_{GB}$ until inversion is reached. The depletion depth is given by

$$D_{\text{core}} = \sqrt{\frac{2\varepsilon s V_{GB}}{q N_d}} \quad (V_{GB} > V_{FB})$$

(2.12)
where $\epsilon_s = 1.03 \times 10^{14}\text{F/m}$ is the permittivity of silicon and $N_d$ is the donor concentration in the $n$-type body.

For $V_{GB} < V_{INV}$, it is assumed that further reduction in $V_{GB}$ results in stronger inversion rather than in more depletion. Thus, the maximum value of the depletion depth is reached

$$D_{\text{core}} = D_{\text{MAX}} \quad (V_{GB} < V_{INV})$$

Strong inversion is achieved when the semiconductor surface contains a density of holes equivalent to that of electrons in the body, i.e., it is as strongly $p$-type as the body is $n$-type. Under this condition, $V_{\text{MAX}}$, the voltage with respect to $V_{FB}$ required to induce $D_{\text{MAX}}$, is given by

$$V_{\text{MAX}} = \frac{2kT}{q} \ln \frac{N_d}{n_i}$$

where $k$ is Boltzmann’s constant, $T$ is absolute temperature (at $T=298^\circ\text{K}$, $kT/q \approx 25\text{mV}$), and $n_i$ is the intrinsic carrier concentration ($\approx 10^{10}\text{cm}^{-3}$ at $T=298^\circ\text{K}$). Therefore, $D_{\text{MAX}}$ is given by substituting $V_{\text{INV}}$ for $V_{GB}$ into equation (2.12)

$$D_{\text{MAX}} = \sqrt{\frac{2\epsilon_s V_{\text{MAX}}}{qN_d}} = 2\sqrt{\frac{\epsilon_s kT \ln(N_d/n_i)}{q^2 N_d}}$$

With expressions for the depletion depth under different $V_{GB}$ ranges, the total photocurrent of the CPG is readily obtained by the summation of the current components at the core and edge regions

$$I_{\text{ph, cpg}}(V_{GB}) = I_{\text{ph, core}}(V_{GB}) + I_{\text{ph, edge}}$$

It is interesting to note that for $V_{GB} > V_{FB}$, since $D_{\text{core}} = 0$ which leads to $I_{\text{ph, core}}(V_{GB}) = 0$, the above formulation correctly describes the fact that the CPG photocurrent comes solely from the edge region.

Fig. 2.5 depicts the approximate theoretical photocurrent for a $50\mu\text{m} \times 50\mu\text{m}$ CPG across $V_{GB}$ for $V_{\text{BODY}} = 1.5\text{V}$, under $1.7\text{pW}/\mu\text{m}^3$ of 620nm (red) optical illumination. The photocurrent is obtained based on equation (2.7)-(2.16). As $V_{GB}$ reduces, the CPG transitions through various modes of operation, in order, accumulation (A), flat band (B), zero bias (C), depletion (D), and inversion (E). For the typical $n$-body doping
level of $1 \times 10^{16}$ cm$^{-3}$ [32], the value of $V_{FB}$ is $\approx 0.45$V and $V_{MAX} = 0.71$V (from equation (2.14)). Therefore, the flat band condition is reached at $V_{GB} \approx 0.45$V. Beyond this point, the core depletion depth $D_{core}$ starts increasing (according to equation (2.12)) as $V_{GB}$ increases. This lasts for 0.71V to the final value of $D_{MAX}$ = 0.41$\mu$m (from equation (2.15)) at which point inversion is reached. Once the depletion region fully forms, the core provides a substantial component to the overall CPG photocurrent. Although the change in the depletion region depth contributes to wavelength-dependent sensing, this effect does not appear to be significant as can be observed in subsequent experimental results. This depth change is primarily responsible for modulating the photogenerated carrier collection efficiency, after light experience wavelength-dependent absorption in the poly gate. The value for $D_{edge}$ of 1$\mu$m for a typical 0.35$\mu$m process is used [33]. Since it is assumed that approximately 40% of incoming light is attenuated by the various oxide layers over the entire chip, the value of 0.3A/W for $R_{core}$ and $R_{edge}$ in equations (2.9) and (2.10) is used, which corresponds to a 60% quantum efficiency. With $T_{gate}(\lambda = 620$nm$) = 0.85$ (from Fig. 2.3), $A_{core} = 48\mu$m$\times 48\mu$m, $A_{edge} \approx 50\mu$m$\times 1\mu$m$\times 4$, the CPG photocurrent is calculated using equation (2.16) to be 102pA and 418pA when the core is active and inactive, respectively.
2.4 VLSI Implementation

The CPG has been prototyped in a 0.35μm standard CMOS technology as depicted in Fig. 2.6. The fabricated CPG cross-sectional view is illustrated in Fig. 2.7, with $p^+$ and $n^+$ diffusion regions implemented as concentric ring structures. The entire CPG is fabricated in an $n$-body for isolation of substrate noise and crosstalk from adjacent pixels in an arrayed implementation.

The sensor integrates an on-chip current-to-frequency ADC. The ADC consists of a comparator and a digital counter. The comparator has one input connected to the CPG and the other to a reference voltage. Photocurrent from the CPG is integrated onto its parasitic capacitor, building the voltage across it. If this voltage exceeds the reference voltage, the comparator triggers a reset operation and the photodiode voltage is reset to its dark value. The counter is incremented for every reset. Therefore, at the end of the exposure, the counter value is proportional to the light intensity.

Fig. 2.8 depicts the measured photocurrent of a 50μm×50μm CPG across gate voltages for blue (450nm), green (520nm), and red (620nm) input illumination provided by LEDs, with full width at half maximum (FWHM) of 20nm. For each color, approximately $3 \times 10^{13}$ photons/sec/cm$^2$ were incident on the photodiode surface. Current measurements are obtained by a semiconductor parameter analyzer. To highlight the relative change in the current, the results are normalized to one at $V_{GB} = 1.8$V. As $V_{GB}$ changes from a high voltage to a low voltage, the CPG core gradually becomes
active, leading to increased photocurrent. As depicted in Fig. 2.8, the photocurrents across colors change to different extents. For a $V_{GB}$ change from 1.8V to -1.5V ($V_{BODY}$ = 1.5V), the increase in the photocurrent for blue, green, and red illuminations are 50%, 260%, and 350%, respectively. To a first-order approximation, these percentages are due to the absorption of light at specific wavelengths by the polysilicon gate as illustrated in Fig. 2.3, rather than due to a change in the core depletion region depth. The ability of the CPG to sense spectrally is based on this difference in the photocurrents which is the most prominent for $V_{GB}$ between 0 and 0.6V. Therefore, in order to resolve the input illumination at, for example, three wavelengths (i.e., three colors), $V_{GB}$ of
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Figure 2.9: Responsivity (experimentally measured results superimposed on simulated results) for different CPG device sizes: (a) 20\(\mu\)m\(\times\)20\(\mu\)m, (b) 50\(\mu\)m\(\times\)50\(\mu\)m, and (c) 100\(\mu\)m\(\times\)100\(\mu\)m device. B, G, and R denote blue, green, and red light, respectively.

0V, 0.3V, and 0.6V are used for the three measurements required by equation (2.5).

The CPG can be characterized by its responsivity, the ratio of the photocurrent to the input illumination power, commonly expressed in the units of A/W. The responsivity is calculated by dividing the simulated CPG photocurrent by the illumination power collected within the total CPG area, including both the core and edge regions.

Fig. 2.9 depicts measured and simulated CPG photocurrents for three device sizes: 20\(\mu\)m\(\times\)20\(\mu\)m, 50\(\mu\)m\(\times\)50\(\mu\)m, and 100\(\mu\)m\(\times\)100\(\mu\)m. All devices have 1\(\mu\)m edge regions. Both cases where the CPG core region is active (‘ON’) and inactive (‘OFF’) are depicted. Data have been measured by a semiconductor parameter analyser. Photocurrent simulations have been performed in the Crosslight optoelectronic simulator [36]. The simulated structure is depicted in Fig. 2.7. Process parameters used, such as gate thickness \(t_{GATE}\) (0.3\(\mu\)m), \(n\)-body doping \((10^{16}\text{cm}^{-3})\) and \(n\)-body depth (1\(\mu\)m), are
for a typical 0.35μm standard CMOS process [33].

At short wavelengths, the photocurrent is low due to the gate absorption, which attenuates light before it reaches the sensing regions. At long wavelengths, light penetrates deeper into the substrate than the n-body depth, resulting in a reduction in the portion of carriers that can be collected.

The responsivity is low when the core is inactive as illumination power is lost to the core that does not generate any photocurrent. Comparing among the three CPG sizes using Fig. 2.9, unlike the photocurrent which is lower for a small device, the CPG responsivity is higher for a small device. A smaller device has a larger perimeter-to-area ratio, hence a proportionately larger edge region. This edge region is not covered by the light-attenuating gate, therefore it has a higher responsivity than the core region. The higher current output per unit area of a smaller device therefore leads to its higher responsivity.

The gate thickness is typically a fixed parameter for a process, but it is illustrative to analyze the associated tradeoff with responsivity and spectral selectivity. Fig. 2.10 depicts the effect of variation in the gate thickness (e.g., for a process design or due to process variation). Fig. 2.10(a) shows that the responsivity improves as $t_{GATE}$ decreases, since more light can reach the sensing region without being absorbed by the gate.

Fig. 2.10(b) depicts the ratio $R$ of the ON-current to the OFF-current, for the
core being active and inactive, respectively. In order for the CPG to differentiate two wavelengths $\Delta \lambda$ nm apart, the change in the ON-OFF current ratio $\Delta R$ must be non-zero to ensure, for the two-color case, $k_{11}/k_{21} \neq k_{21}/k_{22}$ in equations (2.3) and (2.4). For example, if a change in $V_{GB}$ merely affects responsivity to all wavelengths to the same extent, then $R$ would be a constant across wavelengths, and measuring multiple times for different $V_{GB}$ would not yield additional spectral information. The slope $m = \Delta R/\Delta \lambda$ is thus a representation of the spectral selectivity and is ideally large. It can be observed from Fig. 2.10(b) that $m$ has a high value from 400nm to 650nm, which covers the emission spectra of most fluorescent biomarkers and the visible spectrum. Based on Figs. 2.10(a) and (b), a tradeoff can be observed where a thicker gate translates to better filtering which is essential for spectral selectivity, at the expense of reduced responsivity.

The effect of varying the area covered by the gate relative to the total device area on responsivity and spectral selectivity is shown in Fig. 2.11 for different core and edge dimensions, as defined in Fig. 2.7. Fig. 2.11(a) compares two 100$\mu$m $\times$ 100$\mu$m CPGs and shows that theresponsivity improves as core area decreases, due to reduced light absorption by the gate. However, analogous to the aforementioned tradeoff, Fig. 2.11(b) shows that large gate coverage is essential to good spectral selectivity. In fact, a smaller device, Device III (CORE=48$\mu$m, EDGE=1$\mu$m), outperforms Device I (CORE=50$\mu$m, EDGE=25$\mu$m) in terms of maximizing the slope $m$, suggesting that the spectral selectivity is primarily sensitive to the ratio CORE/EDGE, instead of the absolute size of

Figure 2.11: Simulation results for CPGs with various values of core size and edge width: (a) responsivity, and (b) ON/OFF current ratio.
Given the above analysis, the way in which the CPG differs from conventional depth-based approaches to spectral sensing \cite{25,30} can be readily understood. The vertical dimension, such as diffusion depth or well depth, of depth-based devices \cite{25,30} is of intrinsic importance to their spectral performance since an appropriate absorption depth $D$ is required to be able to sense a particular wavelength. But as CMOS devices scale to deep sub-micron planar dimensions, their vertical dimension is often $<100\,\text{nm}$ \cite{35}, rendering the device only sensitive to very short (possibly non-visible) wavelengths. Whereas, although CPG operation involves the formation of the depletion region under the gate structure, its spectral responsivity is predominately due to the absorption property of the poly-silicon gate, a layer available in any standard CMOS technology requiring no additional cost or fabrication steps. Specifically, in Fig. 2.8, where the CPG core is fully active ($V_{GB} << 0\,\text{V}$), the normalized photocurrents of 1.5, 3.6, and 4.5 for the colors blue, green, and red, respectively, are largely due to the gate absorption and are not a result of sensing at precise depths. This makes the CPG be tolerant to device and technology scaling.

### 2.5 Validation in LED Color Light Measurements

The 0.35$\mu$m prototype depicted in Fig. 2.6 has been tested in light intensity measurements at the blue (450nm), green (520nm), and red (620nm) wavelengths using three current-controlled LEDs for input illumination. In order to measure the intensity at three known wavelengths, according to equation (2.5), an empirical model with nine $k$-coefficients is required. The extraction of $k$-coefficients can be performed as follows. For example, in equation (2.3), to extract $k_{11}$, a known $\phi_1$ serves as the input of the measurement $J_1$ (at $V_{GB1}$). Similarly, for $k_{12}$, a known $\phi_2$ is applied as input for another measurement at $V_{GB1}$. This process is then repeated across all wavelengths and gate voltages. Following the above procedure, only nine measurements are required to determine all nine $k$-coefficients. But it has been found that the modeling accuracy can be improved by simultaneously utilizing multiple combinations of input colored light intensities to solve for the average $k$-coefficients in equation (2.5). After the $k$-
coefficients are obtained once, they are stored and reused for subsequent reconstruction calculations.

To resolve the input to three wavelengths, each input is measured three times using the discrete $V_{GB}$ values of 0V, 0.3V and 0.6V as shown in Fig. 2.8. The raw measurements and the previously obtained model are combined to reconstruct the input using equation (2.5). Fig. 3.12 depicts measured intensities after reconstruction for an illumination that simultaneously contains blue, green, and red light. Figs. 3.12(a)-(c) depict, respectively, measured blue, green, and red components of the input. For each of the three wavelengths, intensities at 0, 100, 500, 1000, and 1500μW/cm$^2$ have been tested, which covers the entire emission intensity range of commonly used biomarkers. In order to evaluate the crosstalk between color channels, for each intensity step, the intensities of the other two colors are swept. For example, in Fig. 3.12(a), the blue intensity is held constant while green and red intensities are varied. This process is repeated five times across the blue intensity levels. Therefore, for the five intensity levels, each against 25 combinations of the other two colors, a total of 125 measurements have been
Figure 2.13: (a) Application of CPG in a fluorescent detection microsystem (QD = Quantum Dot), and (b) fluorescent absorption and emission spectra of quantum dots.

performed. To highlight the reconstructed measured intensity variations, Figs. 3.12(d)-(f) are an alternative representation of Figs. 3.12(a)-(c), respectively. Each error bar contains the data from one plane and depicts one standard deviation from the mean value. The signal-to-noise ratio (SNR) is defined for imagers as $SNR = \frac{\mu}{\sigma}$, where $\mu$ and $\sigma$ are mean and standard deviation of the output calculated over temporal measurements [37]. The peak SNRs as calculated at 1500$\mu$W/cm$^2$ (limited by LED output power) are 34.7dB, 29.2dB, and 34.8dB for the blue, green, and red components, respectively. It is worth noting that the variation $\sigma$ in theory contains both color crosstalk and temporal noise due to the pixel and ADC readout circuits. However, as can be observed in Figs. 3.12(a)-(c), the planes are relatively horizontal, indicating that the measurements of a particular color component have no significant dependency on the intensity of the other colors. The results suggest the variations are largely due to the temporal noise, which can be mitigated by multiple sampling and averaging. Table 2.1 summarizes key chip characteristics.

### 2.6 Validation in Color Fluorescence Measurements

The CPG has been integrated into a microsystem, depicted in Fig. 2.13(a), for validation in the detection of green-emitting quantum dots (gQDs) and red-emitting quantum
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Table 2.1: CPG CHIP CHARACTERISTICS

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technology</td>
<td>0.35μm standard CMOS</td>
</tr>
<tr>
<td>Supply Voltage</td>
<td>3.3V</td>
</tr>
<tr>
<td>CPG Sizes</td>
<td>20^2 μm^2, 50^2 μm^2, 100^2 μm^2</td>
</tr>
<tr>
<td>CPG Edge Region Width</td>
<td>1μm</td>
</tr>
<tr>
<td>Detector Type</td>
<td>p^+–FD/n-body photogate</td>
</tr>
<tr>
<td>Diode Capacitance</td>
<td>≈250fF (50μm^2 CPG)</td>
</tr>
<tr>
<td>Poly-Si Absorption (Sim’ed)</td>
<td>65% at 450nm (α=3.56μm^{-1})</td>
</tr>
<tr>
<td></td>
<td>30% at 520nm (α=1.35μm^{-1})</td>
</tr>
<tr>
<td></td>
<td>15% at 620nm (α=0.45μm^{-1})</td>
</tr>
<tr>
<td>Peak SNR (at 1500μW/cm^2)</td>
<td>34.7dB at 450nm</td>
</tr>
<tr>
<td></td>
<td>29.2dB at 520nm</td>
</tr>
<tr>
<td></td>
<td>34.8dB at 620nm</td>
</tr>
<tr>
<td>Peak Responsivity</td>
<td>0.34, at 570nm (20μm^2 CPG)</td>
</tr>
<tr>
<td></td>
<td>0.30, at 570nm (50μm^2 CPG)</td>
</tr>
<tr>
<td></td>
<td>0.28, at 570nm (100μm^2 CPG)</td>
</tr>
</tbody>
</table>

dots (rQDs). The QD absorption and emission spectra are depicted in Fig. 2.13(b). QDs have a number of unique optical properties that make them useful as fluorescent markers for spectral multiplexing. These properties include broad excitation spectra, greater resistance to photobleaching than organic fluorophores, larger attainable Stokes shifts (>100nm) and size-tunable narrow and symmetrical emission spectra [15]. The CPG is used to spectrally differentiate the green and red fluorescence emissions, essentially performing on-chip emission filtering.


2.6.1 **Fluorescent Contact Sensing Microsystem Setup**

The microsystem, depicted in Fig. 2.13(a), consists of an LED for excitation, an optical emission filter, a fluidic structure, and the CPG sensor for photo detection. Excitation is provided by a 450nm (FWHM = 20nm) LED, the blue channel of the Luxeon K2 LED system [38] used in the LED color light measurements. The excitation light is directed through the optical filter to attenuate the excitation light intensity. A thin-film optical interference filter is chosen for this microsystem. It has been tested in laboratory conditions to provide an optical density of six (i.e., factor of 10^6) excitation light attenuation. The performance of the thin-film filter reduces as scattered light rays deviate from the ideal perpendicular-to-the-surface angle of incidence by more than 20 degrees. This behavior is characteristic of interference filters.

To ameliorate the scattering effects caused by uneven drying of QDs spotted on a surface, the QDs are imaged in the solution phase using a reservoir made from polydimethylsiloxane (PDMS) and glass. For tighter control of the geometry of the QD solution to be sensed, a reservoir is fabricated to contain the solution instead of depositing the QD on a sensing surface, such as a transparent cover slip on the CMOS imager.

To fabricate the reservoir, a cylindrical volume is removed by a metal punch from the PDMS, which is subsequently plasma bonded to a glass cover slip. The dimensions of the cover slips, which forms the bottom of the reservoir, are 22mm (length) × 22mm (width) × 150μm (thick). The bottom of the reservoir is made as thin as possible to minimize the distance between the sample and the focal plane. The 4mm-diameter reservoir with a side wall height of 1mm holds a volume of 10μL.

2.6.2 **Sample Preparation**

Oleic acid capped organic core/shell CdSeS/ZnS based QDs from Cytodiagnostics have been made water-soluble by a ligand exchange reaction with 3-mercaptopropionic acid [5]. The quantum yields of the mercaptopropionic acid capped gQDs and rQDs are 0.19(±0.02) and 0.25(±0.03), respectively. As depicted in Fig. 2.13(b), the peak emission of gQDs and rQDs are at 527nm and 623nm, respectively, with a FWHM of
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Figure 2.14: Background-subtracted calibration curves obtained from single-color measurements of: (a) gQDs, and (b) rQDs.

29nm for both colors of QDs. The molar extinction coefficients are $411400 \text{M}^{-1}\text{cm}^{-1}$ and $350000 \text{M}^{-1}\text{cm}^{-1}$ for gQDs and rQDs, respectively.

2.6.3 Single-Color Quantum Dot Sensing

The fluorescence detection capability of the microsystem has been tested by measuring the emission intensity from various concentrations of gQDs and rQDs separately. Since the emission wavelength of the input illumination (gQDs or rQDs) is known when only one color of QD is present in the solution (i.e., 527nm for gQD or 623nm for rQD, but not both), emission intensity is the only quantity to be measured. As a result, for single-color experiments, the CPG functions as a regular non-spectral sensor, i.e., mathematical reconstruction is not necessary.

The calibration curves for rQDs and gQDs are depicted in Fig. 2.14(a) and (b), respectively. Each curve involves the detection of binary-weighted QD concentrations from $3 \mu \text{M}$ down to $24 \text{nM}$, for a total of eight concentration steps. Each error bar denotes one standard deviation from four measurements. Error bars are symmetrical on a linear scale and are included for all data points but in some cases are too small to be visible on the logarithmic plot. The higher response from the detector in case of rQDs as compared to gQDs is due to a higher molar absorptivity coefficient of rQDs as compared to gQDs at the 450nm excitation wavelength (see excitation spectra of rQDs and gQDs in Fig. 2.13(b)).
To determine the detection limit, the noise statistics of the background are first measured. To account for the optical characteristics of the PDMS-glass structure, an empty reservoir filled with a 50mM borate buffer (pH 9.25) solution is utilized in the measurement of the background signal. The mean and standard deviation of the background signal are 50 and 3.4 sensor output codes or LSBs, respectively, from 32 measurements. For each calibration curve, the background, which is attributed mainly to scattering of the excitation light, is subtracted from the measurements to determine the QD emission signal. For chemical measurements, the detection limit is conventionally defined as three standard deviations ($3\sigma$) above the mean of the background signal. In this case, $3\sigma = 10.2$ sensor output codes, which translates to a detection limit of approximately 24nM for both colors of QDs, with a sample volume of 10μL.

### 2.6.4 Simultaneous 2-Color Quantum Dot Sensing

As discussed previously, the need for spatial registration for multiplexed detection can be avoided by means of spectral multiplexing, which is based on the detection of multiple emission wavelengths. In order to evaluate and demonstrate the multi-color detection capability of this microsystem, the simultaneous measurement of gQD and rQD concentrations in a mixture of QDs has been performed.

Green QDs and red QDs are first prepared into four concentrations of 0nM, 375nM, 750nM, or 1500nM. The QDs are then mixed to form 16 solutions with unique ratios of gQD/rQD concentrations. Fig. 2.15 depicts measurements of gQD and rQD concentrations through the sensing of green and red fluorescence emissions from the 16 solutions. For example, solution S7 contains 750nM and 375nM of gQD and rQD, respectively.

The process of spectral sensing for QDs is similar to that of the LED color light measurements. To invoke the spectral sensing capability of the CPG, the input is sampled at multiple CPG gate voltages. In this case of detecting two colors, two gate voltages are used ($V_{GB1} = 0V$, $V_{GB2} = 0.6V$, for $V_{BODY}$ at 1.5V). To improve detection accuracy, eight photocurrent measurements are performed at each $V_{GB}$. The measurements are then averaged and the background subtracted. The set of two re-
Figure 2.15: Measurements of gQD and rQD concentrations by sensing of green and red fluorescence emission. Results from 16 solutions containing both gQD and rQD are plotted. For example, solution S7 contains 750nM and 375nM of gQD and rQD, respectively. Error bars are from four measurements and denote one standard deviation.
resulting averages at $V_{GB1}$ and $V_{GB2}$ then enter the reconstruction algorithm to solve for the gQD and rQD concentrations. This process of measurement and reconstruction is repeated four times to obtain the error bars depicted in Fig. 2.15.

It can be observed from Fig. 2.15 that the standard deviations of the background signals at zero input concentrations are $\sigma_g \approx 40\text{nM}$ and $\sigma_r \approx 30\text{nM}$ for gQD and rQD, respectively. Based on the $3\sigma$ definition, the detection limits of 120nM and 90nM are achieved for gQD and rQD, respectively. Since 10$\mu$L of sample volume is used for each measurement with detection limits in terms of concentration at 120nM and 90nM, the microsystem is able to detect 1.2pmol and 0.9pmol of gQD and rQD, respectively.

Larger error bars are observed in the quantum dot concentration measurements (Fig. 2.15) compared to the LED color light measurements (Fig. 3.12). This can largely be attributed to two main reasons. First, the test environment of LED illumination tests is much more stable in that the LED intensities are well controlled and the path on which light travels from the LED to the sensor is enclosed to suppress the influence of stray light. In contrast, although chemical laboratory techniques has been strictly followed, there is inevitably variation in the sample preparation. For example, although accurate micro-pipetting techniques has been used, the dilution of QDs into different concentrations introduces error. Second, as the excitation light reaches the uneven surface and the non-homogeneity of the QD sample solution, scattering occurs. Scattering characteristics are dependent on the geometry of the sample solution, which although mitigated by the use of the fluidic reservoir, cannot be completely eliminated.

2.7 Discussion

Table 2.2 compares the proposed sensor to CMOS filterless color sensors reported in the last five years. It is evident that the most popular topologies are the buried double and triple junctions [39-43]. A wide variety of CMOS process nodes have been used, with the latest development in a 0.11$\mu$m technology [39]. Also, various readout techniques have been employed such as time division multiplexing [39], event-based readout [40,41], and current readout [43], for applications ranging from biology to machine vision. For a comprehensive evaluation of the proposed CPG prototype, additional discussions are
As evident from equation (2.3) and (2.4), the CPG response has been modeled with a linear system. Based on Fig. 3.12 good linearity has been experimentally validated up to the light intensity of 1500 μW/cm². To further study linearity, a quadratic model (not reported in this work) has been investigated, such that equation (2.3) expands to

\[ I'_1 = k_{11}φ_1 + k_{12}φ_2 + k'_{11}φ_1^2 + k'_{13}φ_2^2 \]  

(2.17)

where \( k'_{11} \) and \( k'_{13} \) model the nonlinear components, for example, due to nonlinear effects in carrier collection. It is found that the \( k' \)-coefficients are of negligible values for the tested illumination range, which further suggests that nonlinearity is not significant. In the case where the CPG were to operate in a nonlinear range, for example when subjected to very high light input intensities beyond the level typically required for biological applications, two approaches can be used. First, as suggested above, a higher order model can be used where the \( k \)-coefficient matrix is expanded to include nonlinear terms. Second, well-adopted modeling techniques such as binning [44] can be used, where the input space is subdivided into multiple regions such that different local \( k \)-coefficients can be assigned to particular input ranges for an accurate reconstruction.

As with most sensors, the CPG response is susceptible to process variations. However, it is worth noting that, since the \( k \)-coefficients are obtained empirically, process variation can largely be accounted for as it is embedded in the \( k \)-coefficients. For

Table 2.2: CMOS FILTERLESS COLOR SENSOR COMPARATIVE ANALYSIS

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<td>BDJ</td>
<td>BDJ</td>
<td>BDJ</td>
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<td>Color / Depth</td>
<td>Color DVS</td>
<td>Color DVS</td>
<td>Retina</td>
</tr>
</tbody>
</table>

BDJ = Buried Double Junction, BTJ = Buried Triple Junction, DVS = Dynamic Vision Sensor, TDM = Time Division Multiplexing
example, inter-die variations can be mitigated by using die-specific $k$-coefficients.

CMOS optical sensor detection limits can be improved by employing low-light techniques, for example, to reduce dark current [45] or using a dedicated capacitive transimpedance amplifier (CTIA) implemented in the pixel to integrate the photocurrent onto a small capacitor, rather than the photodiode parasitic capacitance [37]. However, these techniques tend to reduce fill factor and increase power consumption.

There are two main sources of error associated with the CPG sensor: the error introduced by the readout circuits and the error resulting from the reconstruction process. For the readout circuits, accuracy is limited by the temporal and fixed pattern noise (FPN) of the imager pixel and ADC. The ADC temporal noise can be improved by low-noise analog circuits or by system-level techniques such as oversampling [46], both at the expense of higher power consumption. FPN can be mitigated by, for example, calibrating for transistor threshold voltage variations [47]. To suppress the effect of temporal noise, multiple sampling has been found to be effective both in model generation and in the actual measurement of samples. To improve detection accuracy, averaging has been utilized in measurements involving chemical samples.

Measurement error is also affected by the properties of the reconstruction algorithm. For further investigation, a sensitivity analysis for the system of equations $I = kx$ can be performed to determine the way in which the accuracy of the solution $x$ is affected by the accuracy of $I$, where $I$ is the set of measurements from the sensor. It is worthy to note that this sensitivity is a property of the $k$-coefficient matrix and is determined by the responsivity of the CPG across wavelengths and gate voltages.

2.8 Conclusion

A CMOS color-sensitive photogate sensor is presented. The CPG employs a polysilicon gate available in a standard CMOS process as an optical filter, thus requiring no external color filter. When applied to fluorescence-based biochemical detection microsystems, the CPG can be utilized to detect and differentiate among the emissions of green-emitting and red-emitting quantum dots at the nano-molar concentration level. The entire detection system utilizes only one long-pass optical filter for
excitation attenuation, not color differentiation. The CPG has been experimentally validated by simultaneously sensing two colors of QDs, verifying its suitability for spectrally-multiplexed concentration measurements of biological analytes. The prototype demonstrates technologies that enable miniaturized, low-cost screening tools for medical diagnostics applications.
Chapter 3

CMOS Color Image Sensor with Dual-ADC Shot-Noise-Aware Dynamic Range Extension

3.1 Introduction and Prior Art

In fluorescent imaging, fluorophore-labeled biological samples can vary widely in the amount of light they output. For example, in hybridization assays, target analyte concentrations in the order of nano- to milli-molar are typical [27,48]. In addition, the fluorescent excitation light intensity is typically orders-of-magnitude higher than that of the fluorescence emission. Coupled with the fact that it is difficult to fabricate ultra-thin yet high-performance optical filters, the detection of fluorescence in a contact imaging microsystem may have to be performed in the presence of inadequately-rejected stray excitation light [8]. Therefore, employing a wide dynamic range (WDR) imager is advantageous as it allows for sensing of the low target specimen concentrations superimposed on a substantial background [49]. This chapter presents a shot-noise-aware dynamic range extension technique for CMOS imagers with a dual-ADC configuration.

Numerous dynamic range (DR) enhancement circuit techniques for CMOS image sensors have been reported. The logarithmic sensor provides a wide DR with a simple circuit implementation but achieves a low overall linearity and poor sensitivity under...
high illumination \[50\]. The multiple-capture sensor provides a wide DR and maintains high linearity but results in SNR drops at the high illumination range \[51\]. The asynchronous self-reset technique, albeit requiring a large pixel area, extends the DR and simultaneously achieves high linearity, SNR, and a sensitivity comparable to that of the active pixel sensor \[52, 53\]. Combined high SNR, DR, and linearity are often primary design requirements for biosensors.

Early asynchronous self-reset based prototypes \[54\] output only the self-reset count but neglected the charge that remains in the integration capacitor, commonly referred to as the well. This residue charge is not read out and introduces an error. To mitigate this shortcoming, residue quantization is introduced to the asynchronous self-reset technique \[52\], analogous to a two-step subranging analog-to-digital converter (ADC). The subranging ADC consists of a self-reset ADC, referred to as a coarse ADC (cADC), which produces the most significant bits (MSBs), followed by a residue ADC, referred to as a fine ADC (fADC), which produces the least significant bits (LSBs). The method of residue quantization in \[52\] is by reusing the in-pixel ADC to also process a signal beyond the full well capacity. Since the fADC and cADC have different input ranges, using the same ADC circuit in the presence of input-dependent shot noise does not lead to an overall noise-optimized design.

We present a CMOS color wide dynamic range image sensor prototyped in a standard 0.35\(\mu\)m CMOS technology. The sensor integrates an 8\(\times\)8 array of pixels utilizing the CMOS color photogate (CPG), with the earlier-generation single-pixel prototypes reported by us in \[49, 55, 56\]. We also present a quantitative analysis that accounts for photon shot noise for the architectural design of a two-step ADC for image sensors. Based on this analysis, the sensor implements a WDR asynchronous self-reset readout architecture that places the residue ADC at the column level. The sensor is experimentally validated through the measurement of color light intensities and through 2D color imaging. It is also integrated into a contact imaging microsystem for sensing fluorescent samples in a microfluidic channel.

The rest of the chapter is organized as follows. Section II discusses the overall VLSI architecture. Section III quantitatively analyzes the two-step ADC VLSI architecture in the presence of shot-noise. Sections IV and V discuss the circuit implementation of
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Figure 3.1: VLSI architecture of WDR color digital pixel sensor prototype.

the prototype and reports experimental results, respectively. Section VI describes the experimental validation in fluorescence imaging of samples in a microfluidic channel. Section VII highlights key observations.

3.2 VLSI Architecture

Fig. 3.1 depicts the chip-level VLSI architecture of the imager. The overall image chip micrograph is depicted in Fig. 3.2. The pixel is schematically depicted in Fig. 3.1(b). Each pixel integrates a 50\(\mu\)m\(\times\)50\(\mu\)m color photogate (CPG) \[56\] for color sensing and a \(p^+/n\)-body photodiode to provide greater sensitivity for short-wavelength (i.e., \(\leq 400\text{nm}\)) monochromatic sensing.

The sensor implements the asynchronous self-reset with the residue readout technique. In this scheme, the photocurrent is first estimated by a coarse ADC and the estimation error is subsequently quantized by a fine ADC and used to improve the accuracy of the final result. In the presented implementation, the asynchronous self-reset cADC is located in the pixel but the fADC is implemented as a column-parallel single-slope ADC. This enables the decoupling of specifications for the two sub-ADCs for noise-optimization described in Section IV. The error that results from coarse analog-to-digital conversion, i.e., the residue charge, is buffered by an in-pixel source follower
before it is fed into the column-level fADC.

Fig. 3.3 depicts key signals within the imager. Analog-to-digital conversion is divided into two phases. Phase 1 is a global operation which begins at the start of the integration time, when the ‘cADC RESET’ signal is asserted to reset the integration node and in-pixel counters. The shutter then closes, feeding the photocurrent into the readout circuit, and the photodiode output voltage $V_{PD}$ raises. If illumination is large enough to exceed the well capacity, the comparator generates a pulse at the node $V_{PULSE}$, which turns on the reset transistor to reset $V_{PD}$. Each reset increments the in-pixel counter by one. The number of resets corresponds to the MSBs of the overall two-step ADC output. At the end of integration time, phase 2 begins. In phase 2, the ‘fADC RESET’ signal is asserted to indicate the start of the residue digitization using single-slope operation. For each conversion, a voltage ramp $V_{RAMP}$ is fed into each column-parallel comparator to be compared against the residue voltage, $V_{PD}$. As $V_{RAMP}$ reaches $V_{PD}$, the fADC counter latches in the current value of the global counter. The result of this phase produces the LSBs of the final output. Since the fADC is implemented as a column-parallel ADC, it goes through the sampled residues in the pixels within a column and digitizes them sequentially. In other words, light
exposure and phase 1 conversion are global operations whereas the phase 2 conversion of residues is performed sequentially.

An on-chip R-2R digital-to-analog converter (DAC) is used to successively generate multiple control voltages for the CPGs (described in Chapter 2) and generate the voltage ramp for the column-parallel single-slope ADCs (described in Section IV).

### 3.3 Shot Noise-Aware WDR Two-Step Imager ADC

The exploitation of photon shot noise to reduce the noise requirement of a multi-ramp single-slope imager ADC has been demonstrated [57]. In this section, shot noise exploitation for the asynchronous self-reset with residue readout WDR architecture is described qualitatively first. A quantitative analysis is then presented for a general two-step imager ADC.
3.3.1 Qualitative Analysis

In Fig. 3.4 the transfer characteristic of the image sensor is plotted on a logarithmic scale. Under the customary assumption that one input photon results in one output electron, the input-referred and output-referred quantities are equivalent and are interchangeable for the purpose of the following analysis. Noise sources depicted in Fig. 3.4 are the photon shot noise and read noise, which consists of thermal and flicker noise of the photodiode and readout circuit. In a conventional active pixel sensor (APS), the sensor output increases linearly with the light intensity until the full well capacity $N_{FW}$ is reached. For an imager with extended DR, the output reaches a higher value, $N_{SAT}$, typically limited by mechanisms that are specific to implementation, e.g., in-pixel memory depth for certain self-reset schemes.

The photon shot noise has a standard deviation equal to the square root of the input light signal, in units of electrons. Because photon shot noise increases with the input, as opposed to the input-independent read noise, it becomes the dominant noise source at higher light intensities. In this part of the input range, the conventional ADC has a better noise performance than is required, i.e., its quantization and thermal noise...
can be increased without decreasing the overall noise performance.

Fig. 3.5 also illustrates the above idea for the asynchronous self-reset cADC and residue fADC architecture depicted in Fig. 3.1. In this architecture, the cADC is only active when the input signal exceeds the full well capacity, $N_{FW}$, at which point, the shot noise is $\sigma_{FW}$. Therefore, since the irreducible shot noise component is already substantial, a cADC noise floor much below $\sigma_{FW}$ is over designed in terms of keeping the combined shot noise and read noise at a reasonable level. Unlike the cADC, the fADC operates from the dark condition to $N_{FW}$. Therefore, the fADC noise floor is ideally minimized. Since the area constraint limits the performance of in-pixel ADCs and that a high performance is required from the fADC, it is implemented in the periphery as a column-level circuit.

### 3.3.2 Quantitative Analysis

This section presents an analysis that can be used as a guideline for the architectural design of the two-step ADC. In order to keep the analysis general to any two-step imager ADC architecture, the formulation is not specific to particular fADC and cADC implementations. Therefore, it is assumed that the noise floors of the ADCs are inde-
pendent of the input amplitude, as most types of ADC exhibits this characteristic.

The analysis begins with the well capacity, which is the amount of charge that a pixel can hold in a single photocurrent integration, given by

\[ N_{FW} = \frac{C_{int}V_{int}}{q} \]  

(3.1)

where \( C_{int} \) and \( V_{int} \) are the integration capacitance and integration voltage, respectively, and \( q \) is the elementary charge. It is worth noting that \( C_{int} \) is in most cases the parasitic capacitance of the photodiode. Since \( C_{int} \) is process dependent and \( V_{int} \) depends primarily on the supply voltage, \( N_{FW} \) is largely determined by the process technology.

The asynchronous self-reset with residue readout scheme can be regarded as a conventional active pixel sensor with dynamic range extension at high illumination via self-reset operation. Since imager ADCs are usually designed such that their quantization noise does not exceed the read noise \([57]\), in this analysis, the fADC root-mean-square (RMS) quantization noise, \( \sigma_{q,f} \), is chosen to be equal to the read noise, \( \sigma_{read} \), i.e.

\[ \sigma_{q,f} = \sigma_{read}. \]  

(3.2)

It is well-established that, for an input that follows a uniform probability distribution function, the RMS quantization noise of an ADC, \( \sigma_q \), can be related to the quantization step size, \( N_{LSB} \), as follows \([58]\)

\[ \sigma_q = \frac{N_{LSB}}{\sqrt{12}}. \]  

(3.3)

Therefore, the fADC quantization step size is given by

\[ N_{LSB,f} = \sqrt{12}\sigma_{q,f}. \]  

(3.4)

From the ratio of the largest to the smallest signal, the resolution of the fADC and cADC can be readily formulated. The number of bits for the fADC, \( N \), is given by

\[ \frac{N_{FW}}{N_{LSB,f}} = 2^N \Rightarrow N = \log_2 \frac{N_{FW}}{N_{LSB,f}}. \]  

(3.5)

The saturation signal of the entire two-step DR, \( N_{SAT} \), is determined by the maximum input light intensity specification. The number of bits for the cADC, \( M \), is given by

\[ \frac{N_{SAT}}{N_{FW}} = 2^M \Rightarrow M = \log_2 \frac{N_{SAT}}{N_{FW}}. \]  

(3.6)
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The resolution of the two-step ADC is therefore $M + N$ bits.

The analysis next proceeds to obtain an expression for the RMS quantization noise of the cADC, $\sigma_{q,c}$. The standard deviation of shot noise is given by

$$\sigma_{\text{shot}}(N_{\text{sig}}) = \sqrt{N_{\text{sig}}} \quad (3.7)$$

where $N_{\text{sig}}$ is the input photon count. Referring to Fig. 3.5 in order to constraint the cADC quantization noise to an acceptable level, $\sigma_{q,\text{acceptable}}$, a quality ratio $R$ is defined to relate the cADC noise to shot noise, given by

$$R = \frac{\sigma_{q,\text{acceptable}}}{\sigma_{\text{shot}}(N_{\text{sig}})}. \quad (3.8)$$

If $R = 1$, then $\sigma_{q,\text{acceptable}} = \sigma_{\text{shot}}$. Therefore, to ensure that $\sigma_{q,c}$ is below shot noise, $R$ is chosen to be less than unity.

As depicted in Fig. 3.5 since $\sigma_{q,\text{acceptable}}$ has its minimum at $N_{\text{FW}}$ within the range that the cADC self-resets (i.e., $N_{\text{FW}}$ to $N_{\text{SAT}}$) and since $\sigma_{q,c}$ must be smaller or equal to $\sigma_{q,\text{acceptable}}$, $\sigma_{q,c}$ is chosen to be equal to the acceptable noise level evaluated at the full well level, i.e.

$$\sigma_{q,c} = \sigma_{q,\text{acceptable}}(N_{\text{FW}}). \quad (3.9)$$

This guarantees that $\sigma_{q,c}$ is below shot noise for $N_{\text{sig}} \geq N_{\text{FW}}$. At full well, using equations (3.7) and (3.8), $\sigma_{q,c}$ is given by

$$\sigma_{q,c} = R \cdot \sigma_{\text{shot}}(N_{\text{FW}}) = R \cdot \sqrt{N_{\text{FW}}}. \quad (3.10)$$

Using equations (3.3) and (3.10), the quantization step size for the cADC is given by

$$N_{\text{LSB},c} = \sqrt{12} \sigma_{q,c} = \sqrt{12} R \sqrt{N_{\text{FW}}}. \quad (3.11)$$

As a measure of the noise increase, or equivalently the reduction of the noise requirement of the cADC, the factor $F$ is defined, given by

$$F = \frac{\sigma_{q,c}}{\sigma_{q,f}} = \frac{N_{\text{LSB},c}}{N_{\text{LSB},f}} = \frac{\sqrt{12} R \sqrt{N_{\text{FW}}}}{N_{\text{LSB},f}}. \quad (3.12)$$

which is interestingly proportional to the square root of the well capacity. Also, combining equations (3.5) and (3.12), $F$ can be expressed as

$$F = \frac{R \sqrt{12}}{\sqrt{N_{\text{FW}}}} 2^N. \quad (3.13)$$
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which states that once the fine ADC resolution, \( N \), is fixed, the factor \( F \) is inversely proportional to \( \sqrt{N_{FW}} \).

Key performance metrics of the fADC and cADC can then be computed. The dynamic range is defined as the ratio of the largest signal to the smallest detectable signal. The DRs, in dB, for the fADC and cADC are, respectively

\[
DR_f = 20 \log_{10} \frac{N_{FW}}{\sigma_{q,f}}
\]  

and

\[
DR_c = 20 \log_{10} \frac{N_{SAT}}{\sigma_{q,c}}.
\]  

But as shown in Fig. 3.5, the DR of the overall two-step ADC is not merely \( DR_c \) as is the case for a conventional two-step ADC. Rather, the extended DR is involved, given by

\[
DR_{ext} = 20 \log_{10} \frac{N_{SAT}}{N_{FW}}.
\]  

The DR of the overall two-step ADC is given by

\[
DR_{2STEP} = 20 \log_{10} \frac{N_{SAT}}{\sigma_{q,f}} = DR_f + DR_{ext}.
\]  

This DR analysis reveals an important property of the two-step WDR ADC. In the conventional two-step ADC design where shot noise is not taken into consideration, the cADC covers the entire wide DR but with a large step size and the fADC covers a subrange with a small step size. When shot noise is considered, as in this analysis, the cADC noise floor can be raised, which relaxes the DR requirement of the cADC. However, as illustrated in Fig. 3.5, \( \sigma_{q,c} \) still has to be designed to a level below the cADC LSB step size, namely, the full well level. Therefore, the cADC effective number of bits (ENOB) exceeds its actual number of bits. As mentioned previously, if \( \sigma_{q,c} \) is designed to be in the neighborhood of \( \sigma_{q,f} \), the cADC has better noise performance than is required. But, if \( \sigma_{q,c} \) is equal to the full well level, then the final result of the entire two-step ADC is only accurate to the cADC LSB and renders the entire \( N \) bits of the fADC inaccurate.
The signal-to-noise ratios (SNRs) in dB for the fADC in the read noise dominant (low signal) and shot noise dominant (high signal) regimes are, respectively

\[
SNR_{f,\text{low}} = 20 \log_{10} \frac{N_{\text{sig}}}{\sigma_{q,f}} = 20 \log_{10} \frac{\sqrt{12} N_{\text{sig}}}{N_{\text{LSB},f}}
\]  

and

\[
SNR_{f,\text{high}} = 20 \log_{10} \frac{N_{\text{sig}}}{\sigma_{\text{shot}}} = 20 \log_{10} \sqrt{N_{\text{sig}}}
\]

The peak SNR in dB of the fADC is obtained by evaluating \(SNR_{f,\text{high}}\) at the highest fADC input, given by

\[
SNR_{f,\text{peak}} = 20 \log_{10} \frac{N_{\text{FW}}}{\sigma_{\text{shot}}(N_{\text{FW}})} = 20 \log_{10} \sqrt{N_{\text{FW}}}
\]

assuming, as it is typical of imagers, that shot noise dominates at the full well level. Analogously, since \(\sigma_{q,c}\) is designed (from equation (3.9)) to be below shot noise in the extended dynamic range, the imager is shot noise dominant within this range. Hence the SNR in dB in the extended DR is given by

\[
SNR_{\text{ext}} = 20 \log_{10} \frac{N_{\text{sig}}}{\sigma_{\text{shot}}} = 20 \log_{10} \sqrt{N_{\text{sig}}}
\]

which has the same expression as \(SNR_{f,\text{high}}\). Analogously, the cADC peak SNR in dB is given by

\[
SNR_{c,\text{peak}} = 20 \log_{10} \frac{N_{\text{SAT}}}{\sigma_{\text{shot}}(N_{\text{SAT}})} = 20 \log_{10} \sqrt{N_{\text{SAT}}}
\]

Lastly, the peak SNR of the overall two-step ADC is given by

\[
SNR_{2\text{STEP},\text{peak}} = 20 \log_{10} \frac{N_{\text{SAT}}}{\sigma_{\text{shot}}(N_{\text{SAT}})} = SNR_{c,\text{peak}}
\]

which is different from the ratio of the maximum input to the noise at the zero signal level, as is often the case for most types of ADCs. This difference is a direct result of the presence of shot noise. Strictly speaking, the above peak SNR expressions are approximations, as read noise has not been included. But since the peak SNRs are evaluated at the illumination level where shot noise dominates over read noise, the approximations are nonetheless accurate.
As a numerical example of the above quantitative analysis, the following parameters with values typical to CMOS imagers are assumed [59]: $\sigma_{\text{read}} = 5e^-$, $N_{FW} = 10000e^-$, and $N_{\text{SAT}} = 1 \times 10^6e^-$. Assume that the fADC quantization noise is designed to be equal to the read noise, i.e., $\sigma_{q,f} = \sigma_{\text{read}} = 5e^-$. From equation (3.4), $N_{\text{LSB},f} = 17.3e^-$. From equations (3.5) and (3.6), the number of bits for the fADC and cADC are $N = 9.2$ bits and $M = 6.6$ bits, respectively. Suppose the cADC is designed to have a quantization noise comparable to shot noise at the full well input, i.e., $R = 1$. Therefore, based on equations (3.10) and (3.11), it can be computed that $\sigma_{q,c} = 100e^-$ and $N_{\text{LSB},c} = 346e^-$, respectively. From equation (3.12), the cADC noise floor can be raised 20 times ($F = 20$) while not incurring a severe noise degradation. This translates to a power and/or area saving. From equations (3.20) and (3.22), the peak SNRs for the fADC and cADC are estimated to be 40dB and 60dB, respectively. From equation (3.23), the peak SNR of the two-step ADC is 60dB. From equations (3.14)–(3.16), $DR_f$, $DR_c$, and $DR_{\text{ext}}$ are 66dB, 80dB, and 40dB, respectively. Since the overall DR is $DR_f + DR_{\text{ext}} = 106dB$, the fact that shot noise is taken into account relaxed the cADC noise requirement by 26dB. An important benefit of this is a much reduced pixel area for an in-pixel cADC implementation (an asynchronous self-reset ADC is required to be implemented in-pixel). It is also worth noting that since the shot noise magnitude is equal to the square root of the signal magnitude, in this example, after 10000 resets, the equivalent signal is $10000^2 e^-$. This results in a shot noise of $10000 e^-$, which is equal to the full well capacity. Therefore, the entire residue or the entire fADC output consists of noise.

### 3.4 Circuit Implementation

The design of Fig. 3.1 has been fabricated in a 0.35μm standard digital CMOS technology. The 175μm×175μm pixels with a 10% fill factor are tiled to form a 8×8 array for imaging. These dimensions are, for example, suitable for pitch matching to a microarray, i.e., one pixel per microarray spot.
3.4.1 Pixel Circuit Implementation

The pixel readout circuit consists of an electronic shutter, a reset block, and ADC block that implements asynchronous self-reset, the coarse part of the two-step ADC operation. The reset block includes a comparator and a reset transistor. The ADC has a 15-bit linear feedback shift register (LFSR) counter which dominates the pixel area. The choice of 15-bit is to provide a wide dynamic range even without the use of the fADC. This enables the imager to support a high frame rate mode with pixel-parallel A/D conversion.

Referring to the circuit in Fig. 3.1(c) and timing diagram in Fig. 3.3, in the beginning of each integration period, the counter is cleared and the photodiode output \( V_{PD} \) is charged to the reset voltage \( V_{RST} = 0 \) V. The n-body is biased at the voltage \( V_{BODY} = 1.5 \) V. The photocurrent causes \( V_{PD} \) to rise, charging the integration capacitor \( C_{INT} \). When \( V_{PD} \) reaches the comparator reference voltage \( V_{REF} = 1 \) V, the comparator changes state, causing the reset transistor \( M_{RST} \) to turn on, resetting \( V_{PD} \) to \( V_{RST} \). After reset, the comparator output toggles back to the original state. The combination of two toggles generate a pulse with a width that equals the time it takes to reset \( C_{INT} \).

In the absence of circuit nonidealities and ignoring the residue, the resulting pulse train has a pulse frequency proportional to the incident light intensity. The asynchronous self-reset ADC is less sensitive to supply voltage scaling as it effectively represents the light signal by a digital count, rather than a voltage across a capacitor.

The voltage comparator is a two-stage design with large PMOS input transistors to lower thermal and flicker noise. Fig. 3.6 depicts the voltage comparator. The first stage employs cross-coupling to increase the output resistance of the load transistors \( M_1 \) through \( M_4 \). The second stage provides an additional gain. The first stage and the second stage consume \( 18 \mu A \) and \( 10 \mu A \) for the 3.3V supply, respectively. The comparator has a 66 dB simulated DC gain for resolving \( \approx 1 \) mV for a maximum input swing of 2 V. The comparator has a simulated DC gain of 66dB and operates at up to 10MHz.
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3.4.2 Column-Parallel Analog-to-Digital Converters

The column-parallel single-slope ADC of Fig. 3.1(d) digitizes the residue voltage \( V_{PD} \). It consists of a voltage comparator with the same topology as the in-pixel comparator of Fig. 3.6 and a 15-bit binary counter. A global counter is connected to both the on-chip DAC and the column-parallel ADC sub-circuits as shown in Fig. 3.1(a). During analog-to-digital conversion, it increments in order to have the DAC output a ramp voltage \( V_{RAMP} \) to the column-parallel ADCs. As depicted in Fig. 3.3, \( V_{RAMP} \) is compared to the residue voltage \( V_{PD} \). When \( V_{RAMP} \) reaches \( V_{PD} \), the comparator clocks the register to latch in the present global counter value, which is the digital representation of \( V_{PD} \).


Figure 3.8: Schematic of the operational amplifier within the DAC.

### 3.4.3 Digital-to-Analog Converter

The schematic of the on-chip DAC is shown in Fig. 3.7. The DAC is based on the R-2R architecture \[58\], due to the availability of high-precision resistors in the CMOS process used. Unlike the conventional resistance-ratio ladder converter \[58\], the R-2R converter realizes binary-weighted currents with a smaller number of components and with a resistance ratio of only two, independent of the number of bits.

Switches are sized proportionately to accommodate the binary increase in the current level through each branch. The unit resistors \( R_{unit} \) are 5KΩ non-silicided polysilicon resistors, each occupying an area of 24\( \mu m \times 1.1\mu m \). The opamp is based on the two-stage opamp architecture, depicted in Fig. 3.8. PMOS input transistors are used to lower the flicker noise. The DAC occupies an area of 300\( \mu m \times 120\mu m \).

### 3.5 Experimental Results

#### 3.5.1 Pixel Readout Circuit

The experimentally measured transfer characteristic is depicted in Fig. 4.6, obtained from illumination provided by a halogen lamp onto a pixel. The input light intensity is calibrated by an optical power meter (with a detector calibrated for broadband sensing) and is varied by over four orders of magnitude using neutral density (ND) filters. ND filters used are from Thorlabs with optical densities (OD) of 0.3, 0.7, 1.0, 2.0, 3.0, and 4.0 and are combined to provide various degrees of attenuation. Measurements are collected using the 50\( \mu m \times 50\mu m \) CPG which is set to the highest photo sensitivity, \( i.e., \)
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Figure 3.9: Experimentally measured imager output as a function of input intensity.

\[ V_{GB} = 0V \]. At each intensity, 32 measurements are obtained to calculate the average and standard deviation \( \sigma \) of the imager output.

The experimentally measured SNR of the imager output, depicted in Fig. 4.7, is calculated as mean over standard deviation of the output. The input-referred dynamic range is defined as the maximum output over the RMS value of the readout noise \( \sigma \), i.e., the standard deviation of the imager output under dark condition \[59\]. Therefore, the DR is the range between SNR = 0 and the highest signal level in Fig. 4.7 and is measured to be 82 dB, limited by the maximum light intensity achievable by the light source in the high end and the read noise in the low end. In Fig. 4.7 as the input
signal increases, the SNR improves at 20 dB/dec at low illumination where the total noise is dominated by the input-independent noise of the fADC readout circuit. As the number of reset operations increases, the noise in the self-reset loop (e.g., reset noise) accumulates, which reduces the rate of increase of the SNR to approximately 6 dB/dec at very high illumination levels. This increase in cADC noise has also been reported in [52] and must be minimized. As depicted in Fig. 4.7, the peak SNR of 46.2 dB has been measured at the highest input intensity. Fig. 4.7 also depicts a measured number of reset pulses of 200, which is equivalent to a DR increase of 46 dB over the same CMOS image sensor without any DR enhancement, i.e., with the cADC disabled.

3.5.2 Digital-to-Analog Converter

The DAC consumes 1.24mW and achieves 8-bit accuracy with INL and DNL shown in Fig. 3.11(a) and (b), respectively. The opamp within the DAC achieves a simulated DC gain of 69dB and a 3dB bandwidth of 20kHz.

3.5.3 System-Level Validation in Color Light Measurements

The 0.35μm CMOS prototype in Fig. 4.5 has been tested in light intensity measurements at the green (520nm) and red (620nm) wavelengths using two current-controlled light-emitting diodes (LEDs) for input illumination.

In order to measure the intensity at two known wavelengths, according to equations (2.3) and (2.4), an empirical model with four \( k \)-coefficients is required. The extraction of \( k \)-coefficients can be performed as follows. For example, in equation (2.3), to extract \( k_{11} \), a known light intensity \( \phi_1 \) serves as the input of the measurement \( I_1 \) (at
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Figure 3.12: Simultaneous two-color light-emitting diode (LED) illumination measurements: (a) measured color photogate response for two gate-to-body voltages across illumination (circles). A linear model based on $k$-coefficients is superimposed (mesh). (b) Reconstructed intensity of the green (520nm) input component. (c) Reconstructed intensity of the red (620nm) input component.

For $V_{GB1}$. Similarly, for $k_{12}$, a known light intensity $\phi_2$ is applied as an input for another measurement at $V_{GB1}$. This process is then repeated for $V_{GB2}$. Following the above procedure, only $N$ measurements are required to determine all $N$ $k$-coefficients. Additionally, it has been found that modeling accuracy can be improved by simultaneously utilizing multiple combinations of input colored light intensities to solve for the average $k$-coefficients. The $k$-coefficients are obtained only once, and are stored for subsequent reconstruction calculations.

To resolve the input to two wavelengths, each input light is measured two times using $V_{GB1} = 0V$ and $V_{GB2} = 0.6V$. The raw measurements and the previously obtained model are combined to reconstruct the input using equations (2.3) and (2.4). Fig. 3.12 depicts measured intensities after reconstruction for an illumination that simultaneously contains green (520nm) and red (620nm) light. For each of the two wavelengths, intensities of 0, 60, 120, 240, and 300$\mu$W/cm$^2$ have been used. This intensity range has been chosen to match the typical output of fluorescent biomarkers in a microsystem environment [48]. Fig. 3.12(a) depicts measured color photogate response across the illumination range for two gate-to-body voltages. These data are used to determine the $k$-coefficients to create a linear model (depicted as a mesh). In order to evaluate the crosstalk between color channels, for each intensity step, the intensity of the other color is swept. For example, in Fig. 3.12(b), for each of the six green intensities, the red
intensity is varied in six levels. Therefore, based on six intensity steps, each against six intensities of the other color, a total of 36 measurements have been performed. Each error bar contains a sweep across all intensities of the other color and depicts one standard deviation from the mean value. Figs. 3.12(c) is analogous to Figs. 3.12(b) but for the red component of the input light.

The SNR is commonly defined for imagers as \( \text{SNR} = \frac{\mu}{\sigma} \), where \( \mu \) and \( \sigma \) are the mean and standard deviation of the output calculated over temporal measurements for all pixels \([37]\). As shown in Figs. 3.12(b) and (c), the peak SNRs measured at the intensity of 300\( \mu \)W/cm\(^2\) are 24.3dB and 28.5dB for the green and red components, respectively. In a separate high input intensity test (result not shown), the imager achieves a peak SNR of 29.2dB and 34.8dB for green and red light at the intensity of 1500\( \mu \)W/cm\(^2\) (limited by maximum LED output).

### 3.5.4 System-Level Validation in 2D Color Imaging

Although the imager has been designed for fluorescence sensing application rather than for the photographic application, its ability to reproduce an image is evaluated via capturing of a still photographic image. It is an image abbreviating the string ‘University of Toronto’ by the characters ‘U’, ‘o’, and ‘T’, on a black background. The approximately 10 cm \( \times \) 10 cm image is held approximately 0.9m from the lens, which
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focuses the light onto the pixel array. The lens aperture is at F/16 and the integration time is 1 sec under $\approx 200 \mu W/cm^2$ of illumination. The image captured by the proposed sensor is depicted in Fig. 3.13. The entire array of pixels use a global color model or $k$-coefficient matrix. One key advantage of the proposed approach to color sensing is that each pixel produces the entire set of RGB values. Therefore, color interpolation, a process that approximates missing color information from neighboring pixels commonly performed in cameras with a color filter mosaic array, is not required. This eliminates the associated color artifacts.

Table 3.1 summarizes the experimentally measured electrical characteristics of the image sensor prototype depicted in Fig. 3.2.

### 3.6 Validation in Fluorescent Imaging

The color sensor prototype has been evaluated as a part of a fluorescence imaging microsystem to validate its suitability for point-of-care (POC) diagnostic applications. POC devices are becoming increasingly popular as they promise to bring diagnostic technology from the standard laboratory setting to the patient residence to facilitate early diagnosis [4]. Although miniaturization is a key for the development of such devices, for optical transduction such as fluorescence-based detection, a fluorescence microscope is commonly employed. Despite the high sensitivity and selectivity offered by this transduction method and its widespread applications ranging from the detection of nucleic acids, proteins and small molecules, its incorporation into POC devices has been limited due to the limited portability and the high cost of the instrumentation. One emerging technique with the potential to overcome the limitations of a fluorescence microscope is contact imaging [19]. Unlike the conventional fluorescent microscope, in contact imaging as depicted in Fig. 3.14, the object to be imaged is placed in close proximity to the focal plane, eliminating the need for bulky and expensive optics such as a system of lenses and mirrors, which enables miniaturization to realize lab-on-a-chip platforms.

Microfluidic networks offer many advantages for chemical and biological sensing. First, reaction time is greatly shortened, in some cases from hours to minutes [12], as
### Table 3.1: EXPERIMENTAL RESULTS OF WDR IMAGER SENSOR PROTOTYPE

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technology</td>
<td>0.35μm standard CMOS</td>
</tr>
<tr>
<td>Supply Voltage</td>
<td>3.3V</td>
</tr>
<tr>
<td>Chip Power Consumption (30fps)</td>
<td>8mW</td>
</tr>
<tr>
<td>Pixel Power Consumption (30fps)</td>
<td>100μW</td>
</tr>
<tr>
<td>Pixel Size</td>
<td>175μm x 175μm</td>
</tr>
<tr>
<td>Photodetector Type</td>
<td>p⁺–FD/n-body photogate</td>
</tr>
<tr>
<td>Core Area</td>
<td>1.6mm x 1.6mm</td>
</tr>
<tr>
<td>Array Pixel Count</td>
<td>8 x 8</td>
</tr>
<tr>
<td>Integration Capacitance</td>
<td>250fF</td>
</tr>
<tr>
<td>Counter Size</td>
<td>15-bit (33k count)</td>
</tr>
<tr>
<td>Max. Effective Well Capacity</td>
<td>156ke⁻ x 33k (at $V_{REF}$=1V)</td>
</tr>
<tr>
<td>Optical Sensitivity</td>
<td>3.3μW/cm²/level</td>
</tr>
<tr>
<td>Max. Photocurrent</td>
<td>10μA</td>
</tr>
<tr>
<td>Dark Current</td>
<td>0.25 count/sec</td>
</tr>
<tr>
<td>Max. Pulse Frequency</td>
<td>10MHz</td>
</tr>
<tr>
<td>Frames Per Second</td>
<td>0.1-100</td>
</tr>
<tr>
<td>FPN (1σ)</td>
<td>0.38%</td>
</tr>
<tr>
<td>Peak SNR</td>
<td>46dB</td>
</tr>
<tr>
<td>Dynamic Range</td>
<td>82dB</td>
</tr>
<tr>
<td>DAC Power Consumption</td>
<td>1.24mW</td>
</tr>
<tr>
<td>DAC Maximum INL</td>
<td>+0.9/-0.6 LSB</td>
</tr>
<tr>
<td>DAC Maximum DNL</td>
<td>+0.4/-0.4 LSB</td>
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</table>
active delivery by electrokinetic flow can be used to accelerate interactions between molecules over an otherwise slow diffusion-limited process. Secondly, small sample volumes in the nano-liter range can be readily transported and processed by means of microfluidic networks. Thirdly, sensing of samples within a microfluidic channel where the chemical reaction occurs facilitates real-time detection.

Integrating an imager with microfluidics can serve a variety of applications. Spatial imaging of a fluidic channel, for example, can be a method to analyze the result of electrophoresis experiments where the outcome is determined by detecting the distance traveled by dispersed particles relative to a fluid under the influence of an electric field [60].

Quantum dots (QDs) as fluorescent markers exhibit a number of unique optical properties that render them superior than organic fluorescent dyes. These unique properties include: narrow, symmetric and size-tunable emission spectra (full width at half maximum, FWHM of 25-35nm); strong and broad absorption spectra; high quantum yield (>20%) and long life time (>10ns) [61]. As compared to organic fluorophores, QDs have greater resistance to photobleaching that enables long-term monitoring. The broad absorption spectra of QDs allows for multiple colors of QDs to be excited efficiently with a single excitation source which is generally not possible with organic dyes. These properties make QDs ideal as fluorescent biomarkers.
3.6.1 Microsystem Prototype Design

The microsystem prototype consists of a blue LED for fluorescence excitation, an optical filter for excitation rejection, a fluidic structure for holding the sample solution, and the CMOS CPG imager for photo detection. A 100μm-thick, 1.5mm × 1.5mm optical interference filter is used to attenuate the 450nm (FWHM = 20nm) excitation light from the Philips Luxeon K2 450nm (FWHM = 20nm) blue LED. The filter is fabricated using 60 layers of Nb₂O₅ and SiO₂ (by Omega Optical) to the required specification, and optically tested prior to integration with the CMOS die. This approach is chosen over the direct deposition of thin-film layers over the CMOS die to ensure that well-established methods for coating planar substrates can be used during filter fabrication. The filter is a long-pass design with a cut-off wavelength of 510±2nm. The filter has been tested to provide an optical density (OD) of six (i.e., 10⁶ attenuation) at the excitation wavelength of 450nm, with a transmission rate greater than 90% at 520nm and on average greater than 85% from 520nm to 700nm.

The microfluidic device consists of a hybrid of top polydimethylsiloxane (PDMS) cover and bottom glass substrate. The channels are fabricated in PDMS using a soft-lithography (rapid prototyping and replica molding) technique. PDMS base and curing agent are thoroughly mixed in a 10:1 ratio, the mixture has been degassed under vacuum, and then 3g of the mixture are poured onto the microfluidic template and cured in an oven at 120°C for 30min. The cured PDMS cover is peeled off and the inlets and outlets at the ends of each channel are punched out using a 2mm diameter metal bore. The PDMS cover is then air plasma oxidized for 30s at 10.5W and is immediately sealed to a plasma oxidized glass coverslip.

A simplified cross-sectional view of the resulting microfluidic device is depicted in Fig. 3.14. It has 1cm (length) × 250μm (width) × 11μm (height) channels, terminated by an inlet and an outlet on each end. The microfluidic device is subsequently integrated with the CMOS sensor as shown in Fig. 3.15(a). Fig. 3.15(b) depicts an enlarged view of the microfluidic channel passing over the sensor pixel array.
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Figure 3.15: Photograph of the microfluidic device placed over the CMOS sensor die: (a) overall configuration showing entire package cavity, and (b) close-up view of fluidic channel running across the CMOS die.

3.6.2 Fluorescence Contact Imaging Experimental Results

To evaluate the applicability to fluorescence imaging, the proposed microsystem is utilized to image QDs in a microfluidic device. Figs. 3.16 show measured results from fluorescence imaging as captured by the presented CMOS image sensor. Fig. 3.16(a) captures the fluorescence of 2 μM red QDs (peak emission wavelength at 620 nm) in a microfluidic channel. Since only a single color of emission is to be sensed, the CPG functions as a monochromatic detector (i.e., reconstruction is not necessary) and is set to $V_{GB} = 0$ V to maximize sensitivity. The samples have been imaged under an excitation power of approximately 0.5 mW/mm² and an exposure time of 10 sec. The presence of the fluidic network introduces light scattering, which combined with the stray LED output in the filter passband, resulted in a background signal of approximately 250 sensor output codes. To remove the background signal component, the background signal is subtracted from the original image to produce the result in Fig. 3.16(a). Fig. 3.16(b) depicts a background subtracted image of a spot (diameter 1 mm) of 2 μM solution of red QDs. The sample solution is directly spotted on the thin-film filter, and as a result, the sample spot is approximately 100 μm away from the detector due to the thickness of the filter. To highlight the features in the captured images, Fig. 3.16(a) and (b) are intensity-thresholded to produce Fig. 3.16(c) and (d), respectively. Fig. 3.16(c) highlights the fact that the fluorescence intensity is higher on
Chapter 3. CMOS Dynamic Range Extension

Table 3.2: CMOS FLUORESCENCE MICROSYSTEMS COMPARATIVE ANALYSIS

<table>
<thead>
<tr>
<th></th>
<th>This Work</th>
<th>[8]</th>
<th>[20]</th>
<th>[62]</th>
<th>[37]</th>
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<tr>
<td>CMOS Technology</td>
<td>0.35μm</td>
<td>0.35μm</td>
<td>5μm</td>
<td>0.18μm</td>
<td>0.5μm</td>
</tr>
<tr>
<td>Supply Voltage</td>
<td>3.3V</td>
<td>3.3V</td>
<td>5V</td>
<td>1.8V</td>
<td>3.3V</td>
</tr>
<tr>
<td>Array Pixel Count</td>
<td>8×8</td>
<td>128×128</td>
<td>10×10</td>
<td>128×128</td>
<td>132×124</td>
</tr>
<tr>
<td>Pixel Size</td>
<td>175μm×175μm</td>
<td>15μm×15μm</td>
<td>300μm×300μm</td>
<td>7μm×7μm</td>
<td>20μm×20μm</td>
</tr>
<tr>
<td>Photodetector Type</td>
<td>photogate</td>
<td>n+/p-sub</td>
<td>p+/n-well</td>
<td>n+/p-sub</td>
<td>n-well/p-sub</td>
</tr>
<tr>
<td>Excitation Wavelength</td>
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<td>532nm</td>
<td>470nm</td>
<td>345nm</td>
<td>532nm</td>
</tr>
<tr>
<td>Emission Wavelength</td>
<td>620nm</td>
<td>575nm</td>
<td>530nm</td>
<td>447nm</td>
<td>575nm</td>
</tr>
<tr>
<td>Peak SNR</td>
<td>46dB</td>
<td>N/A</td>
<td>N/A</td>
<td>46dB</td>
<td>44dB</td>
</tr>
<tr>
<td>Dynamic Range</td>
<td>82dB</td>
<td>46dB</td>
<td>40dB</td>
<td>≈50dB</td>
<td>≈53dB</td>
</tr>
<tr>
<td>Chip Power</td>
<td>8mW</td>
<td>26.2mW</td>
<td>N/A</td>
<td>N/A</td>
<td>2.37mW</td>
</tr>
<tr>
<td>Chip Output</td>
<td>Digital</td>
<td>Analog</td>
<td>Analog</td>
<td>Analog</td>
<td>Digital</td>
</tr>
<tr>
<td>Integrated Fluidics</td>
<td>Yes, PDMS</td>
<td>No</td>
<td>No</td>
<td>Yes, PDMS</td>
<td>No</td>
</tr>
</tbody>
</table>

the left hand side of the image. This is due to a concentration gradient of the quantum dot solution, which has been injected into the channel from the inlet located on the left of the image. Thresholding has been performed in software but can be readily implemented on-chip [8]. Table 3.2 compares the proposed work to recently reported CMOS fluorescence imaging microsystems.

To evaluate the fixed pattern noise (FPN) of the imager, uniform illumination is applied to the entire pixel array. Fig. 3.17 depicts the histogram of image intensities resulting from uniform illumination. The average intensity is 251.1 counts with a FPN (1σ) of 0.38%.

It is often meaningful to characterize the detection limit by the required sample size, rather than solely by the analyte concentration [24]. Since 25nL of sample volume has been injected into the channel with a 2μM concentration, the microsystem is able to detect 50fmol of rQD fluorophore. This is advantageous as fewer time-consuming polymerase chain reaction (PCR) cycles are needed to bring the concentration of the target DNA to a level that can be detected.
Figure 3.16: Fluorescence imaging of 2μM red quantum dot in solution phase: (a) background subtracted image of sample in a 250μm-wide microfluidic channel, and (b) background subtracted image of 1mm-diameter rQD spot deposited directly on the thin-film filter. (c) and (d) are intensity-thresholded images of (a) and (b), respectively.

Figure 3.17: Histogram of the sensor output under uniform illumination.

### 3.7 Discussion

Spatial resolution is often traded off for SNR and DR in scientific imagers. For example, in a DNA detection biosensor, the pixel is designed to be pitch-matched to an individual spot on a microarray [13]. The presented pixel has a large area of 175μm×175μm. The rather low fill-factor of 10% is due to the inclusion of a digital counter for asynchronous self-reset operation. To improve fill factor, one approach is to implement the comparator and counter on the column level to perform synchronous reset operation, at the expense of lowered SNR due to delayed reset [63].

The CMOS die is housed inside a 400μm-deep cavity within an integrated circuit package. The microfluidic network is suspended on top of the cavity, as depicted in Fig. 3.15(a). The total distance from the sample to the detector, including the
thickness of the reservoir bottom, is approximately 300μm. This distance leads to a reduced photon collection efficiency compared to depositing the fluorophores on the thin-film filter, which is placed directly on the CMOS die surface. This is the reason for the higher intensity in Fig. 3.16(b) as compared to Fig. 3.16(a). The distance also leads to blurring of the images. Aside from introducing optics such as microlenses to focus the image onto the photo detectors, a possible solution is to design the microfluidic device with an additional lower layer that can be extended down into the chip package cavity [64]. Thus, channels can be routed to this layer to bring the sample solution closer to the pixel array.

3.8 Conclusion

A wide dynamic range CMOS color image sensor is presented. The sensor integrates an 8×8 array of color photogates. It exploits the wavelength-dependent optical absorption properties of the polysilicon gate to yield color discrimination on a standard digital CMOS process without an external color filter array. An analysis is presented for the asynchronous self-reset with residue readout ADC architecture where photon shot noise is taken into consideration. An implementation of this architecture is described where the coarse asynchronous self-reset operation and fine residue quantization are performed with separate circuits, on and off the array, respectively, to yield a noise-optimized design. A prototype is fabricated in a standard 0.35μm CMOS process and is validated in color light measurements. Contact imaging of quantum dot nanoparticles within a microfluidic channel validates the prototype in fluorescence-based analyte detection. The prototype demonstrates technologies that enable miniaturized, low-cost bio-sensing for medical diagnostics applications.
Chapter 4

CMOS Digital Image Sensor VLSI Architecture with Split-Comparator 3-T Pixel

4.1 Introduction and Prior Art

In an imager, although analog-to-digital conversion is typically a significant source of power consumption, it is also of critical importance to reduce the power spent on amplification and readout of image data from the individual pixels. This chapter presents a high-density, low-power imager architecture.

In the last decade, there have been major changes in the way pixel information is read out. The passive pixel sensor [65] utilizes a simple switch in the pixel to read out the photodiode integrated charge. It suffers from many limitations, such as high noise, slow readout, and lack of scalability. The addition of an amplifier to each pixel has addressed these problems, resulting in the active pixel sensor (APS) [66,67].

A conventional APS capable of operating at video rate with power consumption in the sub-mW regime has been achieved [68], but significant source follower power consumption is still integral to the design. Readout techniques that eliminate the source follower to reduce power dissipation have been reported [69,71]. These techniques aggressively scale down the supply voltage and require a conversion from the voltage to
the time domain, such as by pulse-width modulation, to maintain an acceptable signal-to-noise ratio (SNR). However, the resultant use of pulse-width modulation generally yields a low frame rate, a low fill-factor, and an increase in peripheral circuit complexity.

A new class of imager VLSI architecture based on the sharing of amplifiers amongst pixels has been recently reported [72, 73]. Although the infrared imager in [72] is capable of high spatial resolution, power consumption is high. The imager prototype in [73] employs a shared-amplifier to bias the pixels for logarithmic operation, achieving reduced power consumption and pixel area compared to [72]. However, the column bus settling time is strongly dependent on the input photocurrent, which limits the frame rate. Although noise and linearity benefits over the conventional APS have been reported in shared-amplifier architectures [72,73], their speed and power advantage has largely been unexplored.

We present a CMOS image sensor with a split-comparator architecture (SCA). Preliminary results of the sensor have been reported in [74]. Power consumption is reduced by replacing the in-pixel buffer of the conventional APS by a part of a comparator. The comparator serves as an in-pixel 1-bit quantizer within a column-parallel comparator-based analog-to-digital converter (ADC), such as the single-slope or successive-approximation ADC. The common transistors of the comparator are shared among pixels in a column, maintaining a compact 3-T pixel organization.

The rest of the chapter is organized as follows. Section II discusses the overall imager VLSI architecture. Section III discusses pixel and comparator circuit implementation. Sections IV presents a performance analysis of the imager architecture. Section V details experimental results. Section VI highlights key observations.

### 4.2 Imager VLSI Architecture

A simplified VLSI architecture of conventional image sensor arrays with a comparator-based ADC is depicted in Fig. 4.1(a). In the beginning of an exposure, the photodiode is reset. After exposure, the voltage across the photodiode parasitic capacitance carries the signal and is read out by the source follower amplifier. Pixels of the same column are output by time-multiplexing to an ADC. For ADC architectures requiring a com-
Figure 4.1: Placement of comparator in an image sensor: (a) in-column, and (b) in-pixel.

Comparator, such as the single-slope and successive-approximation ADC, the comparator is placed in the column circuit after the row-select multiplexer. In this architecture, power is consumed by both the source follower and the comparator. There are several advantages to placing the comparator in the pixel as depicted in Fig. 4.1(b). One key advantage is power reduction. In the conventional architecture, the in-pixel buffer has to drive the large capacitance of the column bus. High speed and settling accuracy specifications impose stringent requirements on the source followers, which translate to a high power consumption.

There are several advantages to placing the comparator in the pixel as depicted in Fig. 4.1(b). The primary advantage is the elimination of the source follower power consumption. In the conventional architecture, the in-pixel buffer has to drive the large capacitance of the column bus. High speed and settling accuracy specifications impose stringent requirements on the source followers, which translate to a high power consumption.
Figure 4.2: Transistor-level column-parallel implementation of (a) conventional active pixel sensor (APS), and (b) split-comparator architecture (SCA). One column is shown for each architecture.

4.3 Pixel and Comparator Circuit Implementation

The conventional 3-T APS pixel as depicted in Fig. 4.2(a) consists of a photodiode, a reset transistor ($M_1$), a select transistor ($M_2$), and a source follower amplifier ($M_3$). The APS shares the biasing portion of the source follower ($M_9$) amongst pixels in the entire column. To provide adequate speed to drive the large capacitance of the column bus, ensuring an acceptable settling time at node $BUS$, the source follower consumes a large quiescent current, $I_{SF}$.

The in-pixel comparator architecture as depicted in Fig. 4.1(a), albeit advantageous, has not gained wide adaptation mainly due to the associated increase in the pixel area. To maintain a small pixel area, alternative approaches must be employed beyond the
straightforward placement of the comparator in the pixel. Inspired by existing shared-amplifier approaches in integrated sensor arrays [72, 73, 75], the presented circuit of the comparator is distributed between both the pixel and the column-level peripheral circuits. As depicted in Fig. 4.2(b), this topology shares transistors $M_4$-$M_7$ among pixels within a column, hence we refer to it as the split-comparator architecture (SCA).

The column-parallel comparator is implemented as two half-circuits, one located in the pixel receiving the photodiode voltage $V_{PD}$ as an input and the other located in the column receiving a reference voltage $V_{REF}$. The pixel can be implemented with only three transistors: a reset switch $M_1$, a row select switch $M_2$, and the positive terminal of the comparator differential pair $M_3$. Pixel rolling readout timing is identical to that of the APS as depicted in Fig. 4.3. At the beginning of each exposure, the reset signal (RST) is asserted to restore the pixel voltage to its dark level. As light reaches the photodiode, the generated photocurrent discharges the parasitic capacitance on node $V_{PD}$ and lowers the photodiode voltage. At the end of photocurrent integration, the pixel voltage held by the parasitic capacitance of the photodiode is directly connected to a comparator-based analog-to-digital converter (ADC) by the assertion of the row select (RS) signal. As an example, for a column-parallel single-slope ADC, a global counter and digital-to-analog converter generate a voltage ramp ($V_{REF}$) to be compared to $V_{PD}$.

Figure 4.3: Timing diagram of the pixel and a single-slope ADC.
As $V_{\text{REF}}$ reaches $V_{PD}$, the comparator output $V_{OUT}$ triggers the column registers to latch in the current counter value, which is a digital representation of the integrated photocurrent. For electronic shuttering, an extra MOS switch is added between the photodiode and $M_3$ in both the APS in Fig. 4.2(a) and the SCA in Fig. 4.2(b).

The comparator must satisfy several design objectives. In Fig. 4.2(b), transistor $M_1$ is of minimum size to minimize area. $M_2$ should be sized to have a low on-resistance. Since large output swing is required from a one-stage topology, $M_6$ and $M_8$ may be sized with non-minimum lengths to provide a higher gain. But for high-density arrays, all in-pixel transistors can be minimum-size for high pixel density. $M_4$ and $M_5$ are sized for high output impedance to increase the gain. $M_7$ is sized to achieve low voltage headroom.

The SCA has two column buses, one at the current mirror node $BUS_2$, and one at the drain of the current source transistor, node $BUS_1$. The parasitic capacitance $C_{gd}$ of $M_3$ creates coupling between the photodiode output node and node $BUS_2$, which has a large capacitance as it is one of the column buses in the SCA. Through the Miller effect, this parasitic capacitance increases the integration capacitance, which leads to a reduction in the imager conversion gain but a higher well capacity. By switching the position of $M_2$ and $M_3$, this Miller capacitance can be reduced, analogous to a cascoded amplifier. Since $M_5$ is diode-connected, the gain between $V_{PD}$ and the drain of $M_5$ is small, which does not lead to a significant Miller effect in practice.

4.4 Performance Analysis

The noise and linearity benefits of eliminating the source follower stage have been previously investigated [72, 73]. This section presents a quantitative analysis of the speed of the split-comparator architecture and compares it against the conventional APS.

In a column-parallel imager implementation, pixels share one or more column bus, which has a large capacitance. The settling behavior of the column bus characterizes the image sensor speed. In the following analysis, the circuits settling time is examined by considering the dominant pole frequency of the APS and SCA imagers, depicted in
Fig. 4.2(a) and Fig. 4.2(b), respectively.

In Fig. 4.2(a) and Fig. 4.2(b), when the row select signal (RS) is asserted, the select transistor $M_2$ is in deep triode, acting simply as a resistor. Since both circuits have this resistance located at the same position and has a reasonably small value, it can be ignored without significantly affecting the comparison between the two topologies.

For the source follower of Fig. 4.2(a), the dominate pole is at node $BUS$ where the large column bus capacitance is located. The dominate pole frequency of the source follower is given by

$$\omega_{APS} = \frac{1}{\tau_{APS}} = \frac{g_{m3}}{C_{BUS}}$$  \hspace{1cm} (4.1)$$

in rad/s, where $\tau_{APS}$ is the time constant of the settling behavior at node $BUS$, $g_{m3}$ is the transconductance of transistor $M_3$ and $C_{BUS}$ is the capacitive load of the column bus of the APS. $C_{BUS}$ is the sum of several parasitic capacitances given by

$$C_{BUS} = N \cdot C_{gs2} + C_{gs8} + C_{gd9}$$  \hspace{1cm} (4.2)$$

where $N$ is the number of pixels in the column, $C_{gs2}$, $C_{gs8}$, and $C_{gd9}$ are the gate-to-source capacitances of $M_2$ and $M_8$, and gate-to-drain capacitance of $M_9$, respectively. For a large $N$, as it is common in a pixel array, $C_{BUS}$ approaches $N \cdot C_{gs2}$. Since for all but one pixel, $M_2$ is OFF, $C_{gs2}$ is the gate capacitance of a transistor in the cutoff regime, given by

$$C_{gs2} = C_{ox} W_2 L_{ov}$$  \hspace{1cm} (4.3)$$

where $C_{ox}$ is the gate oxide capacitance (with the value of $\approx 4.5}\text{fF}/\mu\text{m}^2$ in a $0.35\mu\text{m}$ CMOS technology [58]), $W_2$ is the transistor width of $M_2$, and $L_{ov}$ is the overlap length.

Therefore, assuming no slewing occurs, the column bus settling time for the conventional APS is given by

$$T_{APS} = k \cdot \tau_{APS} = k \cdot \frac{C_{BUS}}{g_{m3}}$$  \hspace{1cm} (4.4)$$

where $k$ is the number of time constants required to guarantee a certain settling precision, given by $p = 1 - 1/e^k$ (e.g., $k = 5 \Rightarrow p = 0.993$ for a $\approx 7$-bit resolution).

For the SCA of Fig. 4.2(b), node $BUS1$ has to settle every time $V_{PD}$ changes. This occurs with the traversing across pixels of different rows, with different photodiode
output voltages. When the select switch is closed, the voltage at node BUS1 settles to \( V_{PD} - V_{GS3} \) driven by the source follower made of \( M_2, M_3 \) and \( M_7 \), analogous to the APS.

It is worth noting that \( M_2 \) and \( M_3 \) are typically both in the OFF state. Therefore, the column bus capacitances \( C_{BUS1} \) and \( C_{BUS2} \) are approximately equal. But to minimize the drain-to-source voltage required to keep \( M_7 \) in saturation, it is often sized with a large width. Compared to the minimum-sized \( M_2 \) and \( M_3 \), it can be up to 100× larger. In addition, \( M_7 \) operates in the saturation region with a \( C_{gd} \) given by \( 1/3 \cdot WLC_{ox} \), where \( W \cdot L \) is the gate area of \( M_7 \). Since \( L >> L_{ox} \) even for a small \( L \), \( C_{gd7} \) can be substantial. Therefore, the dominant pole is associated with node BUS1. Based on this observation, the dominant pole frequency for the SCA is given by

\[
\omega_{SCA} = \frac{1}{\tau_{SCA}} = \frac{g_{m3}}{C_{BUS1}}. \tag{4.5}
\]

The expression of the settling time for the SCA is thus given by

\[
T_{SCA} = k \cdot \tau_{SCA} = k \cdot \frac{C_{BUS1}}{g_{m3}} \tag{4.6}
\]

which, for a large \( N \), is approximately equal to the expression for the APS in equation (4.4). This is an important result as it implies that the speed of the SCA is similar to that of the APS. To keep \( g_{m3} \) constant across equations (4.4) and (4.6), \( I_{SCA} \) can be biased at \( I_{SF} \). This translates to an overall SCA current reduction of \( I_{CP} \) compared to the APS.

It is worth noting that node BUS and BUS1 have to settle every time \( V_{PD} \) changes. For the SCA of Fig. 4.2(b), when the select switch is closed, the voltage at node BUS1 settles to \( V_{PD} - V_{GS3} \) driven by the source follower made of \( M_2, M_3 \) and \( M_7 \), analogous to the APS. If the change in \( V_{PD} \) is large, slewing may occur. The slew rates can be approximated by \( I_{SF}/C_{BUS} \) and \( I_{SCA}/C_{BUS1} \) for the APS and SCA, respectively, which again highlights the fact that both architectures would have a similar speed if \( I_{SCA} \approx I_{SF} \).

To quantify the above analysis, the settling times for the APS and SCA of Fig. 4.2(a) and Fig. 4.2(b), respectively, are simulated with the Spectre simulator using 0.35\( \mu \)m CMOS process models. Fig. 4.4 depicts the simulated column settling time, which in
this simulation is the time taken to settle to 99.5% of the final value. The SCA settling time is approximately 4% longer than that of the APS (for $I_{SF} = I_{SCA} = 1\mu A$). This is as expected and is due to the capacitance of the second column bus, $C_{BUS2}$. In this example, the power saving is 50%. However, for APS designs where $I_{CP} > I_{SF}$, a larger power saving can be achieved. For example, the design in [68] has $I_{CP} = 16\mu A$ and $I_{SF} = 1.25\mu A$, yielding a potential 11.8× power reduction compared to the APS.

Also, to evaluate the scaling of SCA settling time with respect to power consumption, simulations were run for two bias currents. It was found that the settling time at $I_{SCA} = 2\mu A$ is 55% that at $I_{SCA} = 1\mu A$.

As described in Section III, the relative position of $M_2$ and $M_3$ affects the conversion gain of the SCA imager. To quantify this effect, the conversion gain for both configurations have been simulated. It was found that, as expected, the parasitic $C_{gd}$ reduces the conversion gain, although the simulated differences between the two configurations is less than 0.5% in the 0.35μm CMOS technology used, for a large 10pF column capacitance.
4.5 Experimental Measurement Results

A prototype SCA CMOS image sensor utilizing the single-slope ADC architecture has been fabricated in a 0.35\( \mu \text{m} \) standard CMOS technology as depicted in Fig. 4.5. The prototype has a 64\( \times \)64 array of 10\( \mu \text{m} \times 10\mu \text{m} \) pixels, each equipped with an electronic shutter. The chip provides a 7-bit output, which is sufficient for the target SNR. The 33\( \mu \text{m}^2 \) n+/p-sub photodiode has a dark current of 8fA, measured at 25\degree \text{C}. Pixel fill factor is 33\%, which can be further improved by using minimum sized transistors. A saturation voltage of 2V (from a 3.3V supply) provides a full-well capacity of \( \approx 150\text{ke}^- \).

The readout transfer characteristic of the imager is depicted in Fig. 4.6, obtained from input illumination provided by a halogen lamp. Different input light intensities are produced by neutral density (ND) filters and calibrated by an optical power meter. ND filters used are from Thorlabs with optical densities (OD) of 0.3, 0.7, 1.0, and 2.0 and are combined to provided various attenuations. At each intensity, 64 measurements are obtained to calculate the standard deviation \( \sigma \) and the average output depicted in Fig. 4.6.

The SNR of the output, depicted in Fig. 4.7, is defined as the ratio of the mean
to the standard deviation of the output. Fig. 4.7 exhibits the classical characteristics of imagers [68] in that as the input signal increases, the SNR improves at 20dB/dec where the total noise is dominated by read noise. As the input increases further, the shot noise contribution exceeds readout noise and SNR improves at approximately 10dB/dec. The peak SNR of 33.5dB has been measured at the highest input intensity of 100μW/cm². The dynamic range is defined as the maximum output over the RMS readout noise $\sigma$. Based on extrapolation at 20dB/dec, the DR is estimated to be 54dB.

The fixed pattern noise (FPN) is measured to be 1.2% under 80μW/cm² of uniform input illumination. The histogram of the imager output distribution is depicted in Fig. 4.8(a). The prototype has been designed for high-speed operation of 4K frames/s.
Chapter 4. CMOS Split-Comparator Image Sensor VLSI Architecture

Figure 4.8: (a) Experimentally measured imager fixed pattern noise (FPN) obtained under a uniform illumination of 80μW/cm², and (b) test image captured by the 0.35μm CMOS prototype.

(with comparator operation of up to 33MHz). It has an overall measured power consumption of 8mW. Based on 30frames/s operation, power consumption can be scaled down to a simulated value of approximately 100μW.

Fig. 4.8(b) shows a test image captured the prototype chip. The 5cm × 5cm test image is located at a distance of 0.9 meter from the focal plane. A National 8mm focal length, F/1.3-16 aperture lens projects a visual scene onto the focal plane. The exposure time is 1/16 of a second (controlled by the electronic shutter) with the lens aperture set to F/16. Table 4.1 and Table 4.2 summarize the prototype characteristics and compare them with low-power CMOS imagers, respectively.

4.6 Discussion

First, the SCA enables the reuse of transistors within the comparators, thereby the compact 3-T implementation of the conventional APS pixel is maintained, preserving high pixel density. Second, the SCA has approximately the same speed (only 4% slower due to the second column bus) compared to the APS. Lastly, power can be reduced by the consolidation of the APS source follower and comparator currents.

Correlated double sampling (CDS) is a widely-adopted technique for mitigating reset and flicker noise. In the SCA, as digitization begins within the pixel, the 3-T
Table 4.1: EXPERIMENTAL RESULTS OF THE SCA IMAGER SENSOR PROTOTYPE

<table>
<thead>
<tr>
<th>Technology</th>
<th>0.35μm CMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supply Voltage</td>
<td>3.3V</td>
</tr>
<tr>
<td>Array Size</td>
<td>64×64</td>
</tr>
<tr>
<td>Core Area</td>
<td>0.76mm²</td>
</tr>
<tr>
<td>Fill Factor</td>
<td>33%</td>
</tr>
<tr>
<td>Output Resolution</td>
<td>7-Bit</td>
</tr>
<tr>
<td>Frame Rate</td>
<td>30 frames/s</td>
</tr>
<tr>
<td>Power (at 30fps)</td>
<td>8mW</td>
</tr>
<tr>
<td>Pixel Size</td>
<td>10μm×10μm</td>
</tr>
<tr>
<td>Pixel Transistor Count</td>
<td>3-T plus optional shutter</td>
</tr>
<tr>
<td>Photodiode Type</td>
<td>n+/p-sub</td>
</tr>
<tr>
<td>Photodiode Area</td>
<td>10μm×3.3μm</td>
</tr>
<tr>
<td>Dark Current</td>
<td>8fA/pixel (25°C)</td>
</tr>
<tr>
<td>Full-Well Capacity</td>
<td>150ke−</td>
</tr>
<tr>
<td>Saturation Signal</td>
<td>2V</td>
</tr>
<tr>
<td>Dynamic Range</td>
<td>54dB</td>
</tr>
<tr>
<td>FPN</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

pixel is not compatible with conventional CDS techniques where an in-column analog difference amplifier is used. Instead, digital CDS can be employed [77], requiring an additional digital memory to store the reset signal. Substraction of the reset signal from the image signal is performed digitally. As with analog CDS, since two readouts are required for each pixel value, the readout power is approximately doubled. The SCA is compatible with CDS techniques involving in-pixel dual memory (for storing the reset and reset-plus-image values), although these techniques are only used in specialized applications as they result in a larger pixel (e.g., a 30% increase in pixel area for the design in [8]).

The reduction of FPN is also essential for improving image quality. Column-wise
Table 4.2: COMPARATIVE ANALYSIS

<table>
<thead>
<tr>
<th></th>
<th>JSSC’03</th>
<th>ISSCC’08</th>
<th>JSSC’09</th>
<th>This Work</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMOS (μm)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Supply (V)</td>
<td>1.5V</td>
<td>1.35V</td>
<td>3.3V</td>
<td>3.3V</td>
</tr>
<tr>
<td>Array Size (μm²)</td>
<td>176×144</td>
<td>128×96</td>
<td>32×32</td>
<td>64×64</td>
</tr>
<tr>
<td>ADC Type</td>
<td>SA</td>
<td>PWM</td>
<td>SR</td>
<td>SS</td>
</tr>
<tr>
<td>Chip Area (μm²)</td>
<td>4</td>
<td>1.2</td>
<td>9.5</td>
<td>0.76</td>
</tr>
<tr>
<td>Pixel Area (μm²)</td>
<td>5×5</td>
<td>3.3×3.3</td>
<td>25×25</td>
<td>10×10</td>
</tr>
<tr>
<td>Fill Factor (%)</td>
<td>30</td>
<td>18.5</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>Full Well (ke−)</td>
<td>26</td>
<td>–</td>
<td>113</td>
<td>150</td>
</tr>
<tr>
<td>SNR (dB)</td>
<td>25</td>
<td>–</td>
<td>50.9</td>
<td>33.5</td>
</tr>
<tr>
<td>DR (dB)</td>
<td>25</td>
<td>51.3</td>
<td>95.3</td>
<td>54</td>
</tr>
<tr>
<td>FPN (%)</td>
<td>–</td>
<td>0.6</td>
<td>5.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Frame Rate (fps)</td>
<td>40</td>
<td>9.6</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Power (μW)</td>
<td>550</td>
<td>74.1</td>
<td>316</td>
<td>100</td>
</tr>
<tr>
<td>FOM (Mpixel*fps/mA)</td>
<td>2.76</td>
<td>2.15</td>
<td>1.61</td>
<td>4.07</td>
</tr>
</tbody>
</table>

SS = Single Slope, SA = Successive Approximation, SR = Self Resetting

FPN, *i.e.*, variation across columns, is due to the mismatch among column-parallel registers in both the APS and the SCA. Row-wise FPN for the SCA is due to the mismatch among the two comparator differential input transistors within a column, whereas the row-wise FPN for the APS is due to mismatch among the source followers within a column. Both architectures have a worst case mismatch due to device separation of the entire vertical dimension of the array. It is worth noting that when the source-follower and the differential pair are subjected to the same mismatch, their input-referred offset is comparable. Therefore, the APS and SCA have similar mismatch characteristic. FPN can be mitigated in two ways. For example, the imager can implement delta-difference sampling, utilizing additional switches to sample the offset in each column to remove column-wise threshold voltage variations. Alternatively, FPN can be stored and subtracted digitally off the focal plane.
4.7 Conclusion

A CMOS image sensor with comparator-based column-parallel ADCs has been presented. A single column-parallel comparator is split between pixels and the ADC in the column. This results in a compact 3-T pixel implementation with the minimum transistor count equivalent to that of a conventional 3-T pixel. The in-pixel source follower is eliminated, which translates to a power reduction over the conventional active pixel sensor.
Chapter 5

CMOS Spectrally-multiplexed
FRET-on-a-chip for DNA Analysis

5.1 Introduction and Prior Art

The CMOS color photogate technique (Chapter 2) and the dynamic range extension technique (Chapter 3) have been combined to realize a DNA detection microsystem. This chapter presents the application-level experimental validation of this microsystem and begins with a brief review of DNA detection using optical transduction.

DNA-based sensing technologies interrogate selective hybridization of complementary DNA sequences and are conventionally designed for parallel detection of multiple nucleic acid targets [2].

Although the conventional microarray technology employs spatial registration to achieve unprecedented parallelism, it suffers from disadvantages such as additional processing required to print spots on a surface and the associated spatial variation in terms of the quality of the probe immobilization across discrete spots [2][17]. Also, the hybridization chemistry requires the washing away of samples after introducing them to the probes. This renders real-time sensing difficult, often prohibitive. Therefore only the outcome rather than the dynamics of the biological experiment can be observed. In addition, microarray manufacturing typically involves time-consuming processes and a laboratory environment. They often offer much greater capabilities, such as parallelism,
than required for many in-field applications such as pathogen detection [3].

An alternative to spatial registration is spectral multiplexing. In fluorescence-based DNA detection, different DNA target sequences can be tagged with fluorophores that emit light at different wavelengths, which can be concurrently detected and distinguished. Unlike other spectroscopic techniques, such as Raman spectroscopy, where continuous fine spectral resolution is required, fluorescent imaging requires spectral differentiation among only a few discrete wavelengths [20,37].

Despite the high sensitivity and selectivity offered by the optical detection method, its incorporation into point-of-care (POC) devices has been limited due to the limited portability and high cost of the instrumentation, as a fluorescence microscope is often required today. One emerging technique with a potential to overcome the limitations of a fluorescence microscope is contact imaging [19]. Unlike the conventional fluorescent microscope, in contact imaging as depicted in Fig. 5.1, the object to be imaged is placed in close proximity to the focal plane. In this technique, the resolution of the imaging system is in the order of the pixel size. This technique eliminates the need for bulky and expensive optics such as a system of lenses and mirrors, which enables miniaturization to realize lab-on-a-chip platforms.

In this chapter, a low-cost CMOS spectrally-multiplexed contact imaging microsystem for DNA analysis is presented. The core of the microsystem is a CMOS color sensor.
photogate (CPG) sensor. The pixel consists of the CPG to differentiate among fluorescent biomarkers emission bands and a current-to-frequency analog-to-digital converter (ADC) for digital readout. Only one optical filter to attenuate the excitation light is required. The sensor is prototyped in a standard digital 0.35μm CMOS technology and experimentally validated in the simultaneous detection of two DNA targets, spinal muscular atrophy disease and the E.coli bacteria.

The remainder of the chapter is organized as follows. Section II discusses the DNA detection chemistry used in this work. Sections III and IV present a mathematical model and a prototype of a fluorescent contact imaging system, respectively. Section V reports experimental validation of the biosensor in fluorescence-based DNA detection. Section VI highlights key observations.

5.2 DNA Detection Chemistry

In order to appreciate the requirements and functionality of the microsystem, the chemistry and the schematics of the assay design for a multiplexed detection of nucleic hybridization are depicted in Fig. 5.2. To interrogate the probe-target hybridization event, the assay employs multi-color colloidal semiconductor nanocrystals or quantum dots (QDs) as energy donors and targets labeled with black hole quenchers (BHQs) as acceptors in the Förster resonance energy transfer (FRET) process. FRET is a mechanism that transfers energy between two molecules through space dipolar coupling. A donor molecule may transfer energy to an acceptor molecule, over a distance typically less than 10nm, through non-radiative dipole-dipole coupling [80].

QDs exhibit a number of unique optical properties that are advantageous for spectral multiplexing. These unique properties include: narrow, symmetric and size-tunable emission photoluminescence spectra (full width at half maximum, FWHM, 25-35nm); strong and broad absorption spectra; high quantum yields (>20%) and long life times (>10ns) [6]. As compared to organic fluorophores, QDs have greater resistance to photobleaching that enables long-term monitoring. The broad absorption spectra of QDs allows for multiple colors of QDs to be excited efficiently with a single excitation source which is not possible with organic dyes. These optical properties of QDs make them
Figure 5.2: Förster resonance energy transfer (FRET) based assay design for spectrally-multiplexed detection of DNA hybridization using multi-color QDs and black hole quencher (BHQ) labeled DNA targets. (top) Green-emitting QDs (gQDs) and red-emitting QDs (rQDs) are conjugated with SMN1 and uidA probes respectively. (bottom) Expected changes in the emission of gQDs and rQDs after hybridization. Note: a single excitation source can be used to excite both colors of QDs.
suitable for optical multiplexing and as donors for FRET-based applications [80].

In the proposed assay, green-emitting QDs (gQDs) and red-emitting QDs (rQDs) are conjugated with the SMN1 and uidA probes, respectively. The survival motor neuron 1 (SMN1) sequence is a diagnostic of neurodegenerative disease called spinal muscular atrophy. The uidA sequence is a marker for *E. Coli* bacteria. SMN1 targets are labeled with BHQ1 and uidA targets are labeled with BHQ2. Hence, the FRET pairs relevant to this work are gQDs/BHQ1 (donor/acceptor) and rQDs/BHQ2 (donor/acceptor). Upon introduction of labeled targets, the hybridization event brings the acceptors (BHQ1 and BHQ2) in close proximity to the QDs, which allows the BHQs to absorb a portion of the QD emission via the FRET process. This absorption of energy reduces or quenches the QD emission. This serves as the analytical signal to be detected. A higher target concentration implies more BHQs are present, resulting in a reduction in the overall observable QD emission signals.

The DNA probe and target sequences used are from Integrated DNA Technologies [81] with the following nucleobase composition for SMN1 and uidA, respectively:

**SMN1 probe:** DTPA-5'-ATT TTG TCT GAA ACC CTG T-3'

**SMN1-BHQ1 target:** BHQ1-3'-TAA AAC AGA CTT TGG GAC A-5'

**uidA probe:** DTPA-5'-CTT ACT TCC ATG ATT TCT TTA ACT-3'

**uidA-BHQ2 target:** BHQ2-3'-GAA TGA AGG TAC TAA AGA AAT TGA-5'

where DTPA denotes dithiol phosphoramidite, a chemical moiety used to immobilize the DNA probe sequences on the surface of QDs. Since a donor QD and an acceptor BHQ form a FRET pair for detecting a particular target DNA analyte, it is possible to employ different colors of the QD/BHQ FRET pair to detect multiple target DNA analytes concurrently. This approach is referred to as spectral multiplexing.

Unlike a typical fluorophore that emits light at longer wavelengths upon optical excitation, the BHQs or dark quenchers absorb excitation energy but dissipate the energy of excitation via vibrational relaxation, hence have no associated emission spectra [82]. The absorption and emission spectra of the two FRET pairs used in this work, and
Figure 5.3: Measured absorption and emission spectra of the two FRET pairs used in this work. (a) green QDs donor with BHQ1-labeled acceptors (peak AB = 534nm) and (b) red QDs donor with BHQ2-labeled acceptors (peak AB = 583nm). The spectral overlaps are shown as the shaded area.

The corresponding spectral overlap is shown in Fig. 5.3. Fig. 5.3(a) and (b) depict gQDs donor with BHQ1-labeled acceptors and rQDs donor with BHQ2-labeled acceptors, respectively. The spectral overlap for the two FRET systems is denoted by the shaded area. The spectral overlap integrals for the gQDs/BHQ1 (donor/acceptor) and rQDs/BHQ2 (donor/acceptor) pairs are $3.2 \times 10^{-10} \text{cm}^6 \text{mol}^{-1}$ and $5.9 \times 10^{-10} \text{cm}^6 \text{mol}^{-1}$, respectively.

### 5.3 Fluorescent Contact Imaging Microsystem

To enable small-form-factor, point-of-care DNA analysis, a fluorescent contact imaging microsystem can be used to excite the fluorescent markers and quantitatively detect their emission, which is representative of the target concentration. In order to aid microsystem design optimization, a quantitative model of the fluorescent excitation, emission, and detection process is first presented, followed by a description of a microsystem prototype.
Chapter 5. CMOS FRET-on-a-chip for DNA Analysis

5.3.1 Microsystem Model

A physical model has been developed for the fluorescent contact imaging microsystem shown in Fig. 5.1. The microsystem consists of a light-emitting diode (LED) for excitation, an optical filter for excitation light rejection, a fluidic structure to hold the sample solution, and a sensor for photo detection. The sample is assumed to be a solution containing fluorophores. The formulation seeks to estimate the amplitude and signal-to-noise ratio (SNR) of the fluorescence emission light that is measured by the photo sensor outputting a digital signal. Beginning the analysis with the excitation light, the Beer-Lambert Law can be used to describe the absorption of light traveling through a medium, specifically

\[ T = \frac{I}{I_0} = 10^{-\varepsilon c_f l} \] (5.1)

where \( T \) is the transmissivity or fraction of the excitation light that travels through the sample medium, in this case a layer of fluorophores. It is defined as the ratio of the transmitted excitation light \( I \) to the intensities of the incident excitation light \( I_0 \). \( \varepsilon \) is the molar absorptivity of a fluorophore, \( c_f \) is the fluorophore molar concentration, and \( l \) is the length of the light path through the medium, i.e., the thickness of the medium, given by

\[ l = V_f / A_f, \] (5.2)

where \( V_f \) and \( A_f \) are the fluorophore sample solution volume and the fluorophore footprint area that the solution occupies, respectively.

The absorptivity or the fraction of the excitation light absorbed by the fluorophores can be expressed as

\[ F_a = 1 - T. \] (5.3)

The excitation photon rate, \( R_{ex} \), is the rate at which excitation photons are successfully absorbed by the fluorophore sample, given by

\[ R_{ex} = F_a \frac{P_{ex}}{E_{ph}}, \] (5.4)
where $P_{ex}$ is the power of the excitation light that falls onto the fluorophore sample area $A_f$, given by

$$P_{ex} = P_{src} \frac{A_f}{A_{src}},$$

(5.5)

where $P_{src}$ and $A_{src}$ are the power and the total illuminated area of the excitation source, respectively. $E_{ph}$ is the photon energy of the excitation light, given by

$$E_{ph} = \frac{hc}{\lambda_{ex}},$$

(5.6)

where $h$ is Planck’s constant, $c$ is the speed of light and $\lambda_{ex}$ is the wavelength of the excitation light.

The rate of the fluorophore emission light that reaches the photo sensor pixel area can be estimated by

$$R_{em} = \eta_{cp}\eta_{filter}\eta_{dye}R_{ex},$$

(5.7)

where $\eta_{cp}$ is the fluorescent emission collection efficiency of the pixel, $\eta_{filter}$ is the filter transmissivity at the emission light wavelength, and $\eta_{dye}$ is the quantum yield of the fluorophore. $\eta_{cp}$ is given by

$$\eta_{cp} = \eta'_{cp}A_{pix},$$

(5.8)

where $\eta'_{cp}$ is the fluorescent emission collection efficiency per unit area and $A_{pix}$ is the pixel area.

The photodetector current can be expressed as

$$I_{pd} = qk_{pd}\eta_{ff}R_{em},$$

(5.9)

where $q$ is the electron charge, $k_{pd}$ is the responsivity of the photodetector which is temperature-dependent, and $\eta_{ff}$ is the fill factor of the sensor pixel. The voltage developed at the end of the integration time $T_{int}$ at the output of a direct-integration photodetector can be expressed as

$$V_{pd} = \frac{I_{pd}T_{int}}{C_{pd}},$$

(5.10)

where $C_{pd}$ is the pixel integration capacitance, often implemented by the photodetector parasitic capacitance.
The voltage $V_{pd}$ is often digitized by an ADC. For a self-reset based current-to-frequency ADC\footnote{55}, the digital output is given by

$$D_{out} = \frac{V_{pd}}{V_{ref} - V_{rst}},$$

where $V_{ref}$ and $V_{rst}$ are the reference voltage and reset voltage, respectively. Finally, the SNR of the emission signal is given by

$$SNR(dB) = 20\log\left(\frac{D_{out}}{\sigma_{out}}\right),$$

where $\sigma_{out}$ is the standard deviation of $D_{out}$ at the background level ($i.e.$, when $R_{em} = 0$). This standard definition of SNR assumes noise is independent of the input amplitude. This assumption is valid for a read noise dominate case, for example, an imager fabricated in a standard CMOS technology sensing a fluorescence signal typically of a low intensity\footnote{37,83}. $\sigma_{out}'^2$ is the noise power, which is given by

$$\sigma_{out}'^2 = \sigma_e^2 + \sigma_{ne}^2,$$

where $\sigma_e^2$ and $\sigma_{ne}^2$ are the noise power components attributed to sensor electronics and non-electronic mechanisms, respectively. $\sigma_e^2$ consists of noise sources such as detector dark noise, readout circuit thermal and flicker noise, and ADC quantization noise. Noise associated with other non-electronic mechanisms of the microsystem includes various well-studied effects such as randomness in excitation scattering\footnote{84}, autofluorescence\footnote{85}, and non-specific absorption\footnote{86}. The detection limit is conventionally defined\footnote{87} as the signal that is equal to $3\sigma_{out}'$, $i.e.$, the signal at which SNR=3 (9.54dB).

To summarize, equations (2), (5), and (7) are associated with the chemistry and optics of the microsystem, whereas equations (9)-(11) are associated with VLSI circuit implementation. Equations (5.11) and (5.12) are utilized to estimate the amplitude and SNR of the observed fluorescence signal as it relates to various microsystem parameters.

For the measurement of noise parameters, $\sigma_e^2$ can be obtained by directly illuminating the sensor pixel by a controlled light source whose intensity is calibrated to the level that produces $D_{out}$. The output noise $\sigma_{out}'$ can be obtained by using a non-fluorescent buffer solution to represent the zero signal ($i.e.$, $R_{em} = 0$) and captures the effects of...
5.4 Microsystem Prototype

Fig. 5.4 is a photograph of the prototyped microsystem. The microsystem consists of a blue LED for excitation, an optical emission filter, a fluidic structure for holding the sample solution, and the CMOS CPG sensor for photo detection.

5.4.1 Filter

The optical filter chosen for the microsystem is a discrete thin-film interference filter for attenuating the excitation light from the Philips Luxeon K2 450nm blue LED excitation (FWHM = 20nm) [38]. It has been designed, fabricated (Omega Optical) [88], and optically tested prior to integration with the CMOS die. This approach is chosen over the direct deposition of thin-film layers over the CMOS die to ensure that proven methods for coating planar substrates can be used during filter fabrication. Direct thin-film deposition over the CMOS die involves higher costs due to complications in adjusting the fabrication process to compensate for the temperature and material differences between the surface of the optical filter and the CMOS die. The encapsulation
Chapter 5. CMOS FRET-on-a-chip for DNA Analysis

![Figure 5.5: Measured thin-film filter transmission characteristics.](image)

of bond wires for protection during the coating process in the direct filter deposition method requires additional costs.

In the proposed microsystem, a 100μm-thick, 2.5cm×2.5cm optical filter is diced into several smaller 1.5mm×1.5mm pieces to match the size of the CMOS pixel array. Fabricating multiple filters by dicing a single filter significantly reduces the unit cost. The interference filter is fabricated using 60 layers of Nb2O5 and SiO2. These materials are selected for their durability and optical properties. The coatings are deposited onto a 100μm-thick microsheet of fused silica substrate by vapor deposition. The coated substrate is then cut with a diamond saw to the size required to cover the pixel array. Finally, the diced filter is attached to the CMOS die.

The long-pass interference filter has a cut-off wavelength of 510±2nm. The cut-off wavelength is chosen to transmit the emitted light from the gQDs and rQDs, with the peak emission wavelengths of 520nm and 620nm, respectively. The transmission of the filter is greater than 90% at 520nm and on average greater than 85% from 520nm to 700nm, as depicted in Fig. 5.5. The filter has been tested under laboratory conditions to provide an optical density (OD) of six (e.g., 10^6 attenuation) at the excitation wavelength of 450nm for an angle of incidence of up to 20 degrees from the normal.
5.4.2 Fluidic Structure

To ameliorate the scattering effects caused by uneven drying of the samples, the QDs are imaged in the solution phase as opposed to solid phase. For a tight control of the geometry of the QD solution to be sensed, a micro-reservoir is fabricated to contain the solution instead of depositing the QD on a surface, such as a glass slide.

To fabricate the reservoir, a circular volume is removed by a metal punch from a piece of polydimethylsiloxane (PDMS), which is subsequently plasma bonded to a glass cover slip. The dimensions of the cover slip, which forms the bottom of the reservoir, are 22mm (length) \times 22mm (width) \times 150\mu m (thickness). The bottom of the reservoir is made as thin as possible to minimize the distance between the sample and the focal plane. The 4mm-diameter reservoir, with a side wall height of 1mm, holds a sample volume of approximately 10\mu L.

5.4.3 Microsystem Model Validation

As a form of validation, the physical model is compared with experimental results. In particular, the model has been used to describe the effect of varying the excitation power on the sensor output of the prototype depicted in Fig. 5.4. Fig. 5.6 depicts the sensor output from measuring the emission of 10\mu L of 2\mu M red (620nm) QD solution. The values of model parameters used are enumerated in Table 5.1. Four measurements are performed at 10mW, 20mW, 30mW, and 40mW of blue LED excitation power. Sensor output has been calculated for several pixel collection efficiencies \eta_{cp} and from correlating the model with measurements, it is found that \eta_{cp} is approximately 6%.

5.5 System Validation in DNA Detection

The microsystem integrating the prototype chip in a 0.35\mu m standard CMOS technology has been verified through both single-target (single-color) DNA detection and simultaneous multi-target (spectrally-multiplexed) DNA detection.
Chapter 5. CMOS FRET-on-a-chip for DNA Analysis

Table 5.1: MICROSYSTEM MODEL PARAMETERS AND PHYSICAL CONSTANTS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Model Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\epsilon$</td>
<td>molar absorptivity of rQDs at $\lambda_{ex}$</td>
<td>$1.00 \times 10^8$</td>
<td>$\text{M}^{-1}\text{m}^{-1}$</td>
</tr>
<tr>
<td>$V_f$</td>
<td>rQD sample volume</td>
<td>$1 \times 10^{-8}$</td>
<td>$\text{m}^3$</td>
</tr>
<tr>
<td>$A_f$</td>
<td>rQD sample area</td>
<td>$4 \times 10^{-6}$</td>
<td>$\text{m}^2$</td>
</tr>
<tr>
<td>$c_f$</td>
<td>rQD molar concentration</td>
<td>$2 \times 10^{-6}$</td>
<td>$\text{M}=\text{mol}/\text{L}$</td>
</tr>
<tr>
<td>$P_{src}$</td>
<td>power of the excitation source</td>
<td>$40 \times 10^{-3}$</td>
<td>$\text{W}$</td>
</tr>
<tr>
<td>$A_{src}$</td>
<td>illuminated area of the excitation source</td>
<td>$4 \times 10^{-4}$</td>
<td>$\text{m}^2$</td>
</tr>
<tr>
<td>$\lambda_{ex}$</td>
<td>excitation wavelength</td>
<td>$450 \times 10^{-9}$</td>
<td>$\text{m}$</td>
</tr>
<tr>
<td>$\eta_{ecp}$</td>
<td>fluorescent emission collection efficiency of pixel</td>
<td>0.06</td>
<td>–</td>
</tr>
<tr>
<td>$\eta_{ep}$</td>
<td>$\eta_{ecp}$ per unit area</td>
<td>$1.96 \times 10^6$</td>
<td>$\text{m}^{-2}$</td>
</tr>
<tr>
<td>$A_{pix}$</td>
<td>pixel area</td>
<td>$3.06 \times 10^{-8}$</td>
<td>$\text{m}^2$</td>
</tr>
<tr>
<td>$\eta_{filter}$</td>
<td>filter transmissivity at emission wavelength</td>
<td>0.85</td>
<td>–</td>
</tr>
<tr>
<td>$\eta_{qyc}$</td>
<td>rQD quantum yield</td>
<td>0.25</td>
<td>–</td>
</tr>
<tr>
<td>$k_{pd}$</td>
<td>photodetector responsivity</td>
<td>0.30</td>
<td>$\text{A}/\text{W}$</td>
</tr>
<tr>
<td>$\eta_{fp}$</td>
<td>sensor pixel fill factor</td>
<td>0.10</td>
<td>–</td>
</tr>
<tr>
<td>$T_{int}$</td>
<td>integration time</td>
<td>1.00</td>
<td>$\text{s}$</td>
</tr>
<tr>
<td>$C_{pd}$</td>
<td>pixel integration capacitance</td>
<td>$500 \times 10^{-15}$</td>
<td>$\text{F}$</td>
</tr>
<tr>
<td>$V_{ref}$</td>
<td>ADC reference voltage</td>
<td>0.10</td>
<td>$\text{V}$</td>
</tr>
<tr>
<td>$V_{rst}$</td>
<td>pixel reset voltage</td>
<td>0</td>
<td>$\text{V}$</td>
</tr>
<tr>
<td>$\sigma_{e}$</td>
<td>standard deviation of electronic noise</td>
<td>3.39</td>
<td>LSB</td>
</tr>
<tr>
<td>$\sigma_{ne}$</td>
<td>standard deviation of non-electronic noise</td>
<td>7.72</td>
<td>LSB</td>
</tr>
<tr>
<td>$T$</td>
<td>light transmissivity through fluorophore</td>
<td>0.74</td>
<td>–</td>
</tr>
<tr>
<td>$l$</td>
<td>optical path length through fluorophore</td>
<td>$2.50 \times 10^{-3}$</td>
<td>$\text{m}$</td>
</tr>
<tr>
<td>$F_a$</td>
<td>absorbivity of excitation by fluorophores</td>
<td>0.26</td>
<td>–</td>
</tr>
<tr>
<td>$R_{ex}$</td>
<td>excitation photon rate</td>
<td>$3.24 \times 10^{14}$</td>
<td>$\text{photons/s}$</td>
</tr>
<tr>
<td>$P_{ex}$</td>
<td>excitation power illuminating fluorophore area</td>
<td>$4.00 \times 10^{-4}$</td>
<td>$\text{W}$</td>
</tr>
<tr>
<td>$E_{ph}$</td>
<td>energy per excitation photon</td>
<td>$3.21 \times 10^{-19}$</td>
<td>$\text{J}$</td>
</tr>
<tr>
<td>$R_{em}$</td>
<td>rate of fluorophore emission collected by pixel</td>
<td>$9.14 \times 10^9$</td>
<td>$\text{photons/s}$</td>
</tr>
<tr>
<td>$I_{pd}$</td>
<td>photodetector current</td>
<td>$4.39 \times 10^{-11}$</td>
<td>$\text{A}$</td>
</tr>
<tr>
<td>$V_{pd}$</td>
<td>photodetector output voltage</td>
<td>88.7 (effective)</td>
<td>$\text{V}$</td>
</tr>
<tr>
<td>$D_{out}$</td>
<td>ADC digital output</td>
<td>$887 \left( P_{src}=40\text{mW} \right)$</td>
<td>LSB</td>
</tr>
<tr>
<td>$\sigma_{out'}$</td>
<td>standard deviation of ADC digital output</td>
<td>9.26 (at no fluores.)</td>
<td>LSB</td>
</tr>
<tr>
<td>$SNR$</td>
<td>signal-to-noise ratio of microsystem</td>
<td>$39.6 \left( P_{src}=40\text{mW} \right)$</td>
<td>$\text{dB}$</td>
</tr>
</tbody>
</table>

**Physical Constants**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_A$</td>
<td>Avogadro’s number</td>
<td>$6.02 \times 10^{23}$</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck’s constant</td>
<td>$6.63 \times 10^{-34}$</td>
</tr>
<tr>
<td>$c$</td>
<td>speed of light</td>
<td>$3.00 \times 10^8$</td>
</tr>
<tr>
<td>$q$</td>
<td>electron charge</td>
<td>$1.60 \times 10^{-19}$</td>
</tr>
</tbody>
</table>
5.5.1 Preparation of Quantum Dots

Oleic acid capped organic core/shell CdSeS/ZnS based QDs from Cytodiagnositcs have been made water-soluble by a ligand exchange reaction with 3-mercaptopropionic acid. The quantum yields of the mercaptopropionic acid capped gQDs and rQDs are 0.19(±0.02) and 0.25(±0.03), respectively. The peak emission of gQDs and rQDs are at 520nm and 620nm, respectively, with FWHM = 29nm for both colors of QDs. The molar absorptivity $\epsilon$ of quantum dots has been measured by absorption spectroscopy using the HP8452A spectrophotometer, with the values of $5.90 \times 10^7 \text{M}^{-1}\text{m}^{-1}$ and $1.00 \times 10^8 \text{M}^{-1}\text{m}^{-1}$ at 450nm for gQDs and rQDs, respectively, which are typical for CdSe QDs.

5.5.2 DNA Hybridization Assays

As depicted in Fig. 5.2, to produce a FRET-based assay design, QDs are conjugated with the DNA probes and DNA targets are labeled with BHQs. The SMN1 and uidA sequences, which are marker sequences for a spinal muscular atrophy disease and for the E. coli bacteria respectively, are utilized as DNA targets. For experiments involving single-color QD solutions, 250nM of gQD-SMN1 probe conjugates are incubated with 0nM, 250nM, 750nM, 1250nM, 1750nM and 2250nM concentrations of BHQ1-labeled SMN1 targets. The hybridization has been done overnight in 50mM borate buffer.
with 250mM NaCl at pH 9.25. Similarly, 250nM of rQD-uidA probe conjugates are incubated with various aforementioned concentrations of BHQ2-labeled uidA targets.

For the multi-color experiments, gQD-SMN1 probe conjugates and rQD-uidA probe conjugates at 250nM concentrations each has been incubated with (0nM, 0nM), (250nM, 250nM), (1250nM, 1250nM), (2250nM, 2250nM), (250nM, 2250nM) and (2250nM, 250nM) concentrations of BHQ-targets. The first number in the parentheses corresponds to the concentration of BHQ1-labeled SMN1 target, whereas the second number corresponds to the concentration of BHQ2-labeled uidA target. The hybridization condition is kept the same as that of single-color hybridization experiments.

5.5.3 Single-Target DNA Detection

Fig. 5.7 shows single-color hybridization experiments with both colors of QDs demonstrating the applicability of the assay and the color photogate to quantitatively detect BHQ-labeled DNA targets via FRET-based quenching of QD emission intensity. A hybridization event between the immobilized probes on the surface of QDs and BHQ labeled targets provided proximity necessary for FRET-based energy transfer between the donors (gQDs and rQDs) and the acceptors (BHQ1 and BHQ2). Figs. 5.7(a) and (b) depict measurements from a 250nM solution of gQD-SMN1 probe conjugates, incubated with 0nM, 250nM, 750nM, 1250nM, 1750nM, and 2250nM of BHQ1-labeled SMN1 targets. Figs. 5.7(c) and (d) depict measurements from a 250nM solution of rQD-uidA probe conjugates incubated with 0nM, 250nM, 750nM, 1250nM, 1750nM, and 2250nM of BHQ2-labeled uidA targets.

Figs. 5.7(a) and (c) depict intensity measurements from the proposed CPG sensor for various DNA target concentrations, for a 1 second integration time. A total of eight measurements were taken for a total integration time of 8 seconds. The error bars show one standard deviation of the variation across the eight measurements. As a reference, Figs. 5.7(b) and (d) depict QD emission intensities measured by the QuantaMaster PTI spectrofluorimeter in the presence of a filter, with an optical density of 1 (i.e., 10× attenuation), to attenuate the excitation. For comparison, the area under the curves in Figs. 5.7(b) and (d) are normalized to the CMOS sensor response in the absence of
Figure 5.7: Single-color hybridization experiments. (a)-(b) FRET based transduction with gQDs for the detection of the survival motor neuron 1 (SMN1) sequence, which is a marker sequence for a spinal muscular atrophy disease, (c)-(d) FRET based transduction with rQDs for the detection of the uidA sequence associated with \textit{E. coli}. (a) and (c) presents the measured response from the proposed sensor, whereas (b) and (d) represent the reference response from the PTI QuantaMaster spectrofluorimeter (as a reference). The area under the curves in (b) and (d) are normalized to the CMOS sensor response in the absence of target DNA (\textit{i.e.}, 0nM concentration) and superimposed onto (a) and (c).
target DNA \( (i.e., 0nM \text{ concentration}) \) and superimposed onto Figs. 5.7(a) and (c).

Since there is only one emission band in single-color experiments, emission intensity is the only quantity to be measured as the wavelength is known. As a result, for single-color experiments, the CPG sensor functions as a regular monochromatic sensor, \( i.e. \), mathematical reconstruction is not necessary.

Fig. 5.7 shows that the measurements made using the CPG sensor correlate well with the measurements made using the spectrofluorimeter. For both QD emission bands, the intensity has been observed to decrease with increasing target concentration. This is as expected since increasing the target concentration allowed for a greater number of acceptors (BHQs) to interact with the donors (QDs), resulting in an increase in FRET-based energy transfer, hence a decrease in the QD emission intensity.

The results of Fig. 5.7 exhibit the expected behavior of exponential reduction in the fluorescence emission intensity of QDs with increasing concentrations of targets. The exponential nature of the response with increasing target concentrations is due to the exponential changes in FRET efficiency with increasing number of acceptors, \( i.e. \), the target concentration \( [91] \).

It is worth noting that since the signal transduction is based on FRET, the quenching of the QD emission intensity only occurs upon the hybridization of targets with probes on the QD surface. As a result, a washing step requiring removal of excess non-hybridized target from the solution is not necessary, which provides an opportunity for real-time detection of hybridization profiles.

It can also be observed from Figs. 5.7(a) and (c) that when the DNA targets are not present \( (i.e., \text{ target concentration is zero}) \), sensor output signals of 200 codes \( (\sigma = 8.0) \) and 230 codes \( (\sigma = 8.6) \) are present for the green and red transduction channels, respectively. Based on the conventional 3\( \sigma \) definition, the detection limits of 170nM and 80nM are achieved for the targets associated with green and red channels, respectively. It is worth noting that the ratio of these detection limits can be predicted by the model developed in Section III as the molar absorptivity of rQD is approximately \( 2 \times \) that of the gQD at the 450nm excitation wavelength. Also, since 10\( \mu \text{L} \) of sample volume is used for each measurement with detection limits of 170 nM and 80 nM in terms of concentrations for the green and red channels respectively, the microsystem
is able to detect 1.7pmol and 0.8pmol of target DNA for the green and red channels, respectively.

### 5.5.4 Simultaneous Two-Target DNA Detection

The single-color green and red QD systems have been spectrally-multiplexed into a multi-color system for the simultaneous detection of SMN1 and uidA targets, with results shown in Fig. 5.8. Fig. 5.8 depicts six trials each with different combinations of two DNA target concentrations. The data shown in Figs. 5.8(a) and (b) are obtained from the DNA bioassays by measuring the response with the proposed sensor and a commercial spectrofluorimeter as a reference, respectively. These trials are two-color experiments demonstrating the ability of the CPG to concurrently detect two different DNA targets via FRET-based quenching of QD emissions. Each solution contains gQD-SMN1 probe conjugates and rQD-uidA probe conjugates at 250nM concentrations each, and are incubated with (0nM, 0nM), (250nM, 250nM), (1250nM, 1250nM), (2250nM, 2250nM), (250nM, 2250nM) and (2250nM, 250nM) concentrations of BHQ1-labeled SMN1 targets and BHQ2-labeled uidA targets (denoted as the first and second number in the parentheses, respectively). The integration time for each trial is 1 second.

To invoke the spectral sensing capability of the sensor, the input is sampled at multiple CPG gate-to-body voltages. In this case of detecting two colors of QD emissions, two values of $V_{GB}$ are used. To improve detection accuracy, for each combination of DNA target concentrations, eight measurements are performed at each of the two gate voltages ($V_{GB_1} = 0\, \text{V}, \quad V_{GB_2} = 0.6\, \text{V}, \quad \text{with} \, V_{BODY} = 1.5\, \text{V}$). The data from each set are then averaged, background subtracted, and fed into the reconstruction algorithm to solve for the input intensities at each of the two wavelength bands that indicate the respective concentrations of two targets in terms of the emission intensities from both colors of QDs.

It is worth noting that the measurements shown in Fig. 5.8(a) are based on measured QD emission intensities expressed in terms of concentrations from the intensity-to-concentration relationships established in Figs. 5.7(a) and (c) for the green and red...
Figure 5.8: Spectrally-multiplexed simultaneous two-target DNA detection. The survival motor neuron 1 (SMN1) sequence is a marker for a spinal muscular atrophy disease and the uidA sequence is a marker for *E. coli*. (a) measured with the proposed sensor, and (b) measured with a spectrofluorimeter (as a reference).
QDs based FRET systems, respectively. For example, for the (0nM, 0nM) trial in Fig. 5.8(a), since the absence of both DNA targets results in no quenching of QD emission in the green and red channels, the CPG sensor output, which measures the QD emission intensity is actually the highest of the six trials.

It has been observed that the QD emission intensity of a certain transduction channel is affected by the degree of quenching in a neighboring channel (e.g., in Fig. 5.8(b), compare trials 2 and 5 for the green channel, and trials 2 and 6 for the red channel). This form of crosstalk is attributed to the effects of non-specific absorption [7] in the bioassay, not the CPG sensor. For example, in trial 5, a large degree of quenching in the rQD emission channel due to the presence of high concentration of BHQ2-labeled uidA target also impacts the gQD emission channel. This results in a signal reduction in the gQD emission channel to a greater extent than in trial 2 despite the presence of the same amount of SMN1 targets in both trials. This accounts for the overestimation in trials 5 and 6 of Fig. 5.8(a). Nonetheless, non-specific absorption can be suppressed by using denatured bovine serum albumin to passivate the surface of QDs [7].

It can be observed from Fig. 5.8(a) that when the DNA targets are not present (i.e., target concentration is zero), the sensor reports the expected averaged result of approximately zero target concentration with \( \sigma \approx 80\text{nM} \) and \( \sigma \approx 70\text{nM} \) for the green and red transduction channels, respectively. Based on the conventional \(3\sigma\) definition, the detection limits of 240nM and 210nM are achieved for the targets associated with green and red channels, respectively. Since 10\( \mu \)L of sample volume is used for each measurement with detection limits in terms of concentration at 240nM and 210nM, the microsystem is able to detect 2.4pmol and 2.1pmol of target DNA for the green and red channels, respectively.

5.6 Discussion

Although the CPG exploits the gate structure in standard CMOS technology, thus requiring no external filter, the poly-gate nonetheless attenuate the incoming light before it is sensed. In the 0.35\( \mu \)m CMOS process, the thickness of the polysilicon gate is approximately 300nm [33], leading to an approximate attenuation of 65%, 30%, and 10%.
and 15% for blue (450nm), green (520nm), and red (620nm) light, respectively. This results in a reduction in sensitivity especially in short (≤400nm) wavelengths. One possible solution is to integrate a standard CMOS photodiode in the pixel, as shown in Fig. 4.5. The p+/n-body photodiode can be used for monochromatic measurements of fluorophores that emits in the blue region, extending the range of fluorophores supported by the microsystem.

In this work, eight samples (N = 8) from each VGB have first been averaged, then used in reconstruction. The advantages of averaging N images as opposed to making measurements at N bias voltages can be studied using a two-color example. In the case of averaging, two bias voltages are used. The computations are in taking averages of N samples, followed by solving a system of two equations. In the case of using multiple bias voltages, the question arises concerning the appropriate choices of the N bias voltages. According to Fig. 2.8, having evenly distributed biases that span across the entire VGB axis would not be a good choice as the use of multiple bias voltages in the ‘flat’ regions (i.e., VGB < 0V and VGB > 0.6V) would yield poor linear-independence in the k-coefficient model. Therefore, the eight samples should be chosen within 0 < VGB < 0.6V. In reconstruction, N equations are solved for N unknowns. Assuming that the integration time per sample is much longer than the computation time, as it is likely the case for biological imaging, both cases have approximately the same total integration time. However, the latter case suffers from two problems. First, it has lower scalability to N. Fitting N bias voltages within 0 < VGB < 0.6V where N is large would require very precise electronics. Second, in general, the algorithmic complexity is much higher for solving a system of N equations (N >> 2) than a system of two equations. Therefore, the first approach is preferred.

In conventional microscopes, light penetrates the filter at normal incidence, where interference filters performs well. However, in contact imaging the fluorescence light reaching the detector covers a wide range of angles of incidence because the light emitters are close to the detector. Although experimental results from the prototype have not shown significant background, it is worth noting that excitation rejection can be further improved by filter structures that can tolerate a wide angle of incidence while offering a high optical density, such as a hybrid interference and absorption filter [92].
The range of non-zero target concentrations explored is 250nM to 2250nM. This corresponds to approximately 1.4 orders of magnitude of dynamic range in terms of detectable DNA target concentration, which is typical of FRET-based systems [80]. Since the nature of the assay involves decrease in QDs fluorescence emission intensity with increasing targets concentration, the 250nM concentration of QD-probe conjugates have been chosen to provide sufficient initial QD fluorescence emission intensity in the absence of BHQ-labeled targets, to be subsequently quenched in the presence of targets. It is worth noting that the dynamic range of the assay is tunable, depending on the concentration of QD-probe conjugates, as previously reported [7]. Hence, target concentrations lower than 250nM or greater than 2250nM can be quantitatively determined by simply changing the concentration of QD-probe conjugates. To illustrate, consider the case that nine DNA probe strands are immobilized on a surface of a single QD in a 250nM QD solution. This system can detect up to a maximum of 2250nM of target strands, because the total number of probe molecules for 250nM of QD is 2250nM. Detecting a higher target concentration is not possible because all probes are bound to targets. Therefore, if 125nM concentration of QD-probe conjugates is used instead of 250nM, the lower limit of detection can be further brought down. However, this also brings down the upper limit of detection, as a smaller number of QD-probe conjugates have a smaller number of probe molecules to hybridize with the targets, as only a certain number of probe molecules can be immobilized on the surface of a QD. On the other hand, if 500nM concentration of QD-probe conjugates is used instead of 250nM, the upper limit of detection can be extended to 4500nM.

It is often meaningful to characterize the detection limit in terms of the required sample size, instead of solely based on the analyte concentration [24]. Considering the 10μL of sample volume used, the proposed microsystem achieves a detection limit for DNA targets at the picomolar level. This is advantageous especially for applications where the extraction or amplification of sample DNA in large quantities is difficult. As a further benefit, reduction in the sample volume allows for samples to be processed by microfluidic devices, which enables miniaturized and integrated systems.
Chapter 5. CMOS FRET-on-a-chip for DNA Analysis

5.7 Conclusion

A spectrally-multiplexed fluorescence detection microsystem for DNA analysis is presented. The microsystem has been quantitatively modeled and prototyped, integrating a CMOS color photogate sensor. The sensor detects and differentiates among the emission light of green and red fluorescent biomarkers without mechanical switching of emission filters. The entire detection system utilizes only one long-pass optical filter for excitation light attenuation. The microsystem has been experimentally verified by the simultaneous detection of two DNA targets with the detection limits of 240nM and 210nM for the SMN1 and uidA sequences. The prototype is an enabling technology for miniaturized, low-cost, and parallel gene-based point-of-care diagnostics.
Chapter 6

Conclusion and Future Work

The objective of this research is to develop a set of imager techniques for low-cost and portable biosensing using standard CMOS technology. This research focuses on enhancing the performance of molecular diagnostic platforms through innovative techniques on the detector device, circuit architecture, and microsystem integration levels. The major challenges on which the thesis focuses have been the high complexity and cost of existing optical sensing apparatus. These shortcomings are indeed the primary limitations of DNA analysis instrumentations today, which prevent their adoption for point-of-care applications.

In Chapter 2, a CMOS color photogate sensor is presented. The wavelength-dependent optical absorption properties of the polysilicon gate is exploited to yield color discrimination on a standard digital CMOS process without an external color filter array. When applied to fluorescence-based biochemical detection microsystems, the CPG can be utilized to detect and differentiate among the emissions of green-emitting and red-emitting quantum dots at the nano-molar concentration level. The entire microsystem utilizes only one long-pass optical filter for excitation attenuation. No optical filter is required for differentiation between the two emission wavelengths. The CPG has been experimentally validated by simultaneously sensing two colors of QDs, verifying its suitability for spectrally-multiplexed concentration measurements of biological analytes.

In Chapter 3, a dynamic range extension technique for CMOS image sensors is
presented. The sensor integrates an 8×8 array of color photogates. An analysis is provided for the asynchronous self-reset with residue readout ADC architecture where photon shot noise is taken into consideration. An implementation of this architecture is described where the coarse asynchronous self-reset operation and fine residue quantization are performed with separate circuits, on and off the pixel array, respectively, to yield a noise-optimized design. A prototype is fabricated in a standard 0.35μm CMOS process and is validated in color light measurements. Contact imaging of quantum dot nanoparticles within a microfluidic channel validates the prototype in fluorescence-based analyte detection.

In Chapter 4, a CMOS image sensor with comparator-based column-parallel ADCs has been presented. A single column-parallel comparator is split between pixels and the ADC in the column. This results in a compact 3-T pixel implementation with the minimum transistor count equivalent to that of a conventional active pixel sensor. The in-pixel source follower is eliminated, which translates to a power reduction over the conventional APS.

In Chapter 5, a spectrally-multiplexed fluorescence detection microsystem for DNA analysis is presented. The microsystem has been quantitatively modeled and prototyped, integrating a CMOS color photogate sensor. The sensor detects and differentiates among the emission light of green and red fluorescent biomarkers without mechanical switching of emission filters. The entire detection system utilizes only one long-pass optical filter for excitation light attenuation. The microsystem has been experimentally verified by the simultaneous detection of two DNA marker gene sequences for a spinal muscular atrophy disease and for the *E. coli* bacteria, to detection limits of 240nM and 210nM, respectively. The prototype is an enabling technology for miniaturized, low-cost, and parallel gene-based point-of-care diagnostic devices.

Potential future research endeavours may include several activities. On the device level, for the CMOS color photogate, multiple device geometries, in particular, the precise size and location of the photogate core and edge regions can be further experimentally explored to improve the sensor spectral response. Although the CPG is capable of differentiating between the 520nm (green) and 620nm (red) quantum dots, further analysis is required to determine the spectral resolution of the device.
Chapter 6. Conclusion and Future Work

On the circuit level, multiple sampling techniques such as correlated double sampling (CDS) \cite{77} can be used to further reduce the reset and fixed-pattern noise of the sensor. Techniques such as the use of per-pixel capacitance transimpedance amplifier (CTIA) can be incorporated to further improve low-light performance, however at the expense of additional power consumption. On the microsystem level, the presented model can be extended to include the physical mechanisms that contribute to chemistry noise. This would allow the co-design and optimization between the assay chemistry and sensor electronics.

In the future, it is envisioned that point-of-care diagnostic devices will play a key role in healthcare. Using CMOS as a foundation in an integrated microsystem has the potential of making rapid, low-cost, high-throughput point-of-care biological testing a reality.
Appendix A

Supplementary Hardware and Software Documentation

A.1 Board Design

To experimentally characterize the presented prototype chip, a 6-layer printed circuit board has been designed using the Altium Designer software. The manufactured board is depicted in Fig. [A.1]. The main components on the board are:

- Altera Cyclone-III 10K 1.8V/3.3V 256-pin BGA field programmable gate array (FPGA) for generating the digital input signals to and acquiring the digital output signals from the chip.
- 44-pin programmable ROM for programming the FPGA.
- 3.3V ultra-low-noise, high-PSRR, low-dropout linear voltage regulators for providing stable supply voltages.
- Octal 12-bit 2.5V DAC for generating voltage biases.
- USB controller for prototype chip-to-PC communication.
- 20MHz oscillator for generating the master FPGA clock.
- Multiple low-noise op-amps for amplifying and buffering signals across the PCB.
Figure A.1: Printed circuit board used to experimentally characterize the fabricated image sensor prototypes.

- Multiple debug pins to monitor the digital signals generated by the FPGA and the chip on the logic analyzer.
- Multiple potentiometers for generating the appropriate bias currents to the chip.
- BNC connector for main power intake.
- JTAG connector for programming the PROM for the FPGA.

### A.2 MATLAB Interface

MATLAB (version 7.0) scripts are used to provide an automated interface between the FPGA and the user. The interface performs the following key tasks:

- Generating the bias voltages and currents for the chip by programming the corresponding DACs.
- Recording and displaying the video frames from the chip.
- In the case of the color photogate, reconstruct the intensities at various wavelengths given measurements at different gate voltages.
Specific MATLAB files are listed as follows:

- **coreA1pixel.m**: For the photogate sensor, this file is run to capture the intensity of the pixel at the centre of the array. The user can specify the number of samples to capture. Various statistics such as mean, standard deviation, and signal-to-noise ratio are calculated and displayed.

- **coreAarray.m**: For the photogate sensor, this file is run to capture an image from the entire array. The user can specify the number of frames to capture.

- **coreB1pixel.m**: For the split-comparator sensor, this file is run to capture the intensity of the pixel at the centre of the array. The user can specify the number of samples to capture. Various statistics such as mean, standard deviation, and signal-to-noise ratio are calculated and displayed.

- **coreBarray.m**: For the split-comparator sensor, this file is run to capture an image from the entire array. The user can specify the number of frames to capture.

- **usbTest.m**: This file is run to perform diagnostics on the USB interface. *dummyData.v* can be used to generate a digital ramp to be displayed by this matlab file on the PC screen.

### A.3 FPGA programming

Quartus II integrated development environment has been used to program the FPGA in order to generate the required signals and configure the chip in different modes of operation. A number of Verilog hardware description language (HDL) scripts perform this configuration task.

- **top.v**: The top-level Verilog module which instantiates all of the required sub-modules.

- **coreA.v**: The master controller for the CMOS photogate sensor. This module defines the scanning of pixels through the entire array, as well as defining the
operation of the pixel, such as reset, integration, and output. This module also defines imager A/D conversion operation.

- **coreB.v**: The master controller for the split-comparator image sensor. This module defines the scanning of pixels through the entire array, as well as defining the operation of the pixel, such as reset, integration, and output. This module also defines imager A/D conversion operation.

- **voltageDAC.v**: Controls the on-board DAC voltages.

- **usbDivider.v**: Defines a clock divider ratio which determines the clock speed of the USB interface. The source clock speed is 20MHz.

- **dummyData.v**: Generates an ascending ramp of digital data for testing the USB interface.

- **startp.v**: Generates the startup sequence for USB communication.

- **datap.v**: Handles the hand-shaking between the MATLAB USB interface and FPGA USB interface.
Bibliography


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