CMOS multimodal sensor based lab-on-a-chip system for personalized bio-imaging diagnosis

Huang, Xiwei

2015

Huang, X. (2015). CMOS multimodal sensor based lab-on-a-chip system for personalized bio-imaging diagnosis. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/62261>

<https://doi.org/10.32657/10356/62261>

NANYANG
TECHNOLOGICAL
UNIVERSITY

**CMOS MULTIMODAL SENSOR BASED
LAB-ON-A-CHIP SYSTEM FOR
PERSONALIZED BIO-IMAGING DIAGNOSIS**

HUANG XIWEI
**SCHOOL OF ELECTRICAL & ELECTRONIC
ENGINEERING**
2014

**CMOS MULTIMODAL SENSOR BASED
LAB-ON-A-CHIP SYSTEM FOR
PERSONALIZED BIO-IMAGING DIAGNOSIS**

HUANG XIWEI

School of Electrical and Electronic Engineering

A thesis submitted to the Nanyang Technology University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2014

Abstract

The world population is rapidly ageing with proportion of people aged 60-year-old and over, growing faster than any other age groups. Considering the current aging society, there is an emerging need to develop the future diagnosis with portable biomedical devices by bio-instrument miniaturization. The recent development of lab-on-a-chip (LOC) technology has provided a promising integration platform of microfluidic channels, microelectromechanical systems (MEMS), and sensors, which allow non-invasive and near-field sensing functions. The standard complimentary metal-oxide semiconductor (CMOS) process allows a low-cost system-on-chip solution to integrate sensors from multiple domains, which has raised many new design challenges.

In this thesis, we have particularly studied the CMOS multimodal sensor for LOC integrated bio-imaging diagnosis system, including: 1) CMOS (capacitive-micromachined-ultrasonic-transducer) CMUT sensor for non-invasive ultrasound imaging towards the glaucoma diagnosis; 2) CMOS (ion-sensitive-field-effect-transistor) ISFET sensor for ion imaging towards the DNA sequencing application; and 3) CMOS optical sensor for microfluidic contact imaging towards the cell detection, recognition, and counting application. We will illustrate the need and application of the three corresponding bio-imaging diagnosis methods as well as design problems addressed when being miniaturized, which can be summarized as follows.

- Firstly, we illustrate one device-level design work using the example of ultrasound imaging. A two-channel analog front-end (AFE) IC for interfacing multi-channel high frequency CMUT array is developed with three-dimensional high resolution imaging capability for glaucoma diagnosis. The main challenge is the process integration between MEMS array and CMOS readout circuit, where flip-chip bonding is deployed. With the use of 30V high-voltage 0.18 μm Bipolar-CMOS-DMOS (BCD) technology, the proposed AFE IC cell is designed to consist of two high-voltage (HV) pulsers in the transmit path, and a shared single low-noise pre-

amplifier in the receiver path for area reduction. The electrical functionality of the proposed AFE IC is characterized in which the HV pulser generates a delay of 16.2ns between the 33ns input trigger pulse and the HV output pulse while driving the load capacitance of 43pF from 0 to 30V. And the low-noise preamplifier achieves over 60dB Ω transimpedance gain with 27.5pA/sqrt(Hz) input referred noise current at 35MHz. A successful pulse-echo acoustic testing is also demonstrated with the developed AFE IC that integrates the CMUT sample in an oil-immersed environment.

- Secondly, we discuss one circuit-level design work using the example of ion imaging. A 64 $\times$ 64 1200fps dual-mode CMOS ion-image sensor is demonstrated with suppressed fixed-pattern-noise (FPN) for accurate high-throughput DNA sequencing. The main challenge of the traditional ISFET-based ion imaging is lack of faulty pH value detection. In this work, we show the solution by pruning sensed data with reference from multi-domain. A dual-mode ISFET sensor is developed, including pH sensing from chemical domain as well as image sensing from optical domain. An ISFET with standard 4T-CMOS image sensor (CIS) pixel structure is proposed and fabricated in standard 0.18 μ m 1P6M CIS process. After addressing physical locations of DNA slices determined by the optical contact imaging, local pH value of one DNA slice can be mapped to its physical address with the accurate correlation, which can significantly improve the DNA sequencing accuracy. Moreover, pixel-to-pixel ISFET threshold voltage mismatch or FPN is reduced by a correlated double sampling (CDS) readout circuit structure that supports both image and pH modes for large-array and high-throughput application. Measurement results show a sensitivity of 103.8mV/pH and FPN reduction from 4% to 0.3% with a readout speed of 1200fps.
- Lastly, we present one system-level design work using the example of microfluidic contact imaging. A microfluidic contact imaging system has been developed with poly-dimethylsiloxane (PDMS) microfluidic channel integrated on top of CMOS image sensor for flowing cell detection, recognition and counting. The main challenge of such a lensless

microfluidic system is how to improve spatial resolution because of no optical lens. To resolve the raw spatial resolution limitation from pixel size, an extreme-learning-machine based single-frame super-resolution processing (ELM-SR) is proposed that can recover high-frequency loss in detected cell contacting images such that flowing cells can be still distinguished for counting. The prototyped lensless microfluidic system obtains less than 8% counting error for absolute number of microbeads; and 0.10 coefficient of variation for cell-ratio measurement of mixed RBC and HepG2 cells in solution.

In this thesis, we have shown a thorough study to explore multimodal CMOS sensors in LOC towards the portable personalized bio-imaging diagnosis system, which could pave the way towards a variety of personalized diagnosis applications such as: 1) CMOS ultrasound sensor for non-invasive human body scanning; 2) CMOS dual-mode ion sensor for portable DNA sequencing; and 3) CMOS contact imaging sensor for point-of-care blood cell tests. Note that the primary novelty of this thesis is the design of CMOS ISFET ion-image sensor (published in IEEE Symposium on VLSI Circuits 2014). As a conclusion, the CMOS multimodal sensor based LOC system has been shown with the great potential to provide the future personalized e-healthcare solution for the coming aging society.

Acknowledgement

The successful completion of my thesis relies on the supports from many people. I am grateful for all their help and caring during my graduate study at NTU.

First and foremost, I would like to express my sincerest gratitude to my advisor, Prof. Yu Hao, for his continuous guidance and support. His research enthusiasm, extensive knowledge, profound vision, problem solving skill, and innovative thinking style have motivated me to overcome all the difficulties throughout my PhD studies, and will continue to drive me in my future career and life.

Besides, I am also deeply grateful to Dr. Yan Mei, for all her detailed guidance, precious advices, and discussions. She is not only a colleague but also a mentor to me. Her industrial experience has taught me how to be professional in doing circuit and system design.

I would like to express my special thanks to Prof. Kang Yuejun and Guo Jinhong, without whom I could never finish the microfluidics related work and biomedical testing. I also want to appreciate Prof. Chang Chip Hong, Prof. Yeo Kiat Seng, Prof. Zhang Wei, Prof. Ramakrishna Kakarala, Prof. Yang Liang, Prof. Siek Liter, Prof. Dennis Sylvester (also at Umich, MI), Prof. Kim Tae Hyoung, Prof. Liu Ai Qun at NTU, and Dr. Je Minkyu (now at DGIST, Korea), Dr. Cha Hyouk-Kyu (now at SNUST, Korea), Dr. Cheong Jia-Hao at Institute of Microelectronics, A*STAR for their help and guidance.

I wish to acknowledge the help from colleagues and friends related to my research projects, Jia Qixiang, Revanth Nadipalli, Dr. Zhang Chun, Jin Xin, Wang Tongxi, Wang Fei, Guo Jing, Wang Xiaolong, Shen Shanlan, Wang Jiacheng, Lim Pei Wern, Qian Hanhua, Nishanth, Xue Peng, Ding Yichen, and Dr. Cheong Wai Chye. I appreciate all administrative staffs in VIRTUS and CHiPES, especially Lim-Tan Gek Eng, Sia Liang Poo, Tay-Teo Boon Ping Dorothy, and Chua Ngee Tat, for their kind support.

I have enjoyed working in this friendly NTU CMOS Emerging Technology Group, and want to thank these group members and friends, Fei Wei, Shang Yang, Wu Wei, Li Sizhen, Cai Deyun, Fu Haipeng, Wang Yuhao, Zhang Yonghao, Wang Kanwen, Xu

Dongjun, Sai Manoj, Song Yang, Wu Sih-Sian, Ma Shunli, Li Peng, Chen Shuai, Xin Guosong, Huang Hantao, Yang Chang, Zhang Wenjuan, and all others. In addition, I want to thank all other colleagues and friends in VIRTUS, CHiPES, IME-A*STAR, SCBE and SCELSE, where I have worked.

I wish to thank all the friends in our football team. The matches we played together will be a good memory to me. And thank those dear friends, Zheng Jun, Li Xingming, Zheng Yin, Yu Xiaofei, Rao Yi, Wu Chong, Liu Zhiqiang, Mao Yingyan, and Chen Lei. Your friendships are invaluable to me.

Finally, my greatest appreciation goes to my dearest Dad, Huang Zhirong, Mom, Zhao Jinfeng, grandparents, my lovely girl Feng Xiaoxian, and all other family members for their endless love and unconditional support. Without their encouragement and devotion, I could not have enough determination to go through all these years.

HUANG, Xiwei

黄汐威

Singapore

July 2014

Acronyms

1P6M	One-Poly Six-Metal
2D	Two-Dimensional
3D	Three-Dimensional
3T	Three-Transistor
4T	Four-Transistor
ADC	Analog to Digital Converter
AFE	Analog Front-End
AIDS	Acquired Immune deficiency syndrome
ART	Antiretroviral Therapy
ASIC	Application Specific Integrated Circuit
BCD	Bipolar-CMOS-DMOS
BSA	Bovine Serum Albumin
CAD	Computer-Aided Design
CCD	Charge-Coupled Devices
CDS	Correlated Double Sampling
CIS	CMOS Image Sensor
CMFB	Common-Mode Feedback
CMOS	Complementary Metal Oxide Semiconductor
CMUT	Capacitive Micromachined Ultrasonic Transducer
CT	Computed Tomography
CV	Coefficients of Variation
DAC	Digital-to-Analog Converter
DMFB	Digital Microfluidic Biochip

DNA	Deoxyribonucleic Acid
DNL	Differential Nonlinearity
DR	Dynamic Range
DSP	Digital Signal Processing
EOF	Electroosmotic Flow
ELM	Extreme Learning Machine
ELM-SR	Extreme-Learning-Machine based Single-frame SR Processing
ENOB	Effective Number of Bits
FBS	Fetal Bovine Serum
FFT	Fast Fourier Transformation
FL	Fluorescent Light Emission
FOV	Field-of-View
FPS	Frame Per Second
FPGA	Field Programmable Gate Array
FPN	Fixed Pattern Noise
FSC	Forward Scattering
FWHM	Full-Width-at-Half-Maximum
GBW	Gain Bandwidth product
GF	GlobalFoundries
HepG2	Human Liver Hepatocellular Carcinoma Cell
HIV	Human Immunodeficiency Virus
HR	High Resolution
HV	High Voltage
IC	Integrated Circuits
INL	Integral Nonlinearity

IPA	Isopropyl Alcohol
ISFET	Ion-Sensitive Field-Effect Transistor
LED	Light Emitting Diode
LOC	Lab-on-a-Chip
LPCVD	Low-Pressure Chemical Vapor Deposition
LR	Low Resolution
LUCAS	Lensless Ultra wide-field Cell monitoring Array platform based on Shadow imaging
MEM	Minimum Essential Media
MEMS	Micro Electro Mechanical System
MRI	Magnetic Resonance Imaging
MSE	Mean Squared Error
MSSIM	Mean Structural Similarity
OFM	Optofluidic Microscope
PBS	Phosphate Buffered Saline
PC	Personal Computer
PCB	Printed Circuit Board
PD	Photo Diode
PDMS	Poly-dimethylsiloxane
PEB	Post Expose Bake
POC	Point-of-Care
PSNR	Peak Signal-to-Noise Ratio
RBC	Red Blood Cell
REFET	Reference Field-Effect Transistor
RSD	Redundant Signed Digit

RX	Reception
SF	Source Follower
S/H	Sample-and-Hold
SL	Soda Lime
SLFN	Single Hidden Layer Feed Forward Neural Network
SNDR	Signal to Noise-plus-Distortion Ratio
SNR	Signal-to-Noise Ratio
SR	Super-Resolution
SRAM	Static Random Access Memory
SROFM	Sub-pixel Resolving Optofluidic Microscope
SS	Single Slope
SSC	Side Scattering
SSIM	Structural Similarity
TIA	Transimpedance Amplifier
TGC	Time Gain Control
TSMC	Taiwan Semiconductor Manufacturing Company
TX	Transmission
USB	Universal Serial Bus
UV	Ultraviolet
VGA	Variable Gain Amplifiers
WHO	World Health Organization

Contents

Abstract	i
Acknowledgement	v
Acronyms	vii
List of Figures.....	xv
List of Tables	xxi
Chapter 1 Introduction.....	1
1.1 Motivation.....	1
1.2 Research Objective and Major Contributions.....	4
1.3 Organization of the Thesis	6
Chapter 2 Background and Literature Review.....	7
2.1 Traditional Bio-Imaging	7
2.1.1 High-resolution Optical Microscope Imaging	7
2.1.2 Non-invasive Ultrasound Imaging.....	9
2.1.3 Problems of Traditional Bio-imaging	11
2.2 Lab-on-a-chip Bio-imaging	12
2.2.1 Lab-on-a-Chip with Microfluidics.....	12
2.2.2 Contact Imaging.....	16
2.2.3 Chemical Imaging.....	21
2.3 CMOS Multimodal Sensor	22
2.3.1 CMOS Image Sensor	22
2.3.2 Multimodal CMOS Image Sensor	23
Chapter 3 LOC Fundamentals – Microfluidic Fabrication and Imaging Modeling	27
3.1 Microfluidics Fabrication.....	27

3.1.1	Photolithography Mask.....	27
3.1.2	SU-8 Mold	28
3.1.3	PDMS Replica	31
3.2	LOC Imaging System Model.....	33
3.2.1	Resolution	35
3.2.2	Dynamic Range.....	37
Chapter 4	CMOS CMUT Sensor for Ultrasound Imaging	39
4.1	CMUT Sensor and Ultrasound Imaging	39
4.2	Ultrasound AFE Architecture	41
4.3	Ultrasound AFE Design Specifications	44
4.4	Circuit Design and Implementation	48
4.4.1	Process Selection	48
4.4.2	High Voltage Pulser	48
4.4.3	Preamplifier.....	51
4.5	Measurement Results	54
4.5.1	AFE IC Characterization.....	54
4.5.2	Acoustic Transmission Testing with AFE IC and CMUT.....	59
4.5.3	Acoustic Pulse-Echo Testing with AFE IC and CMUT	61
4.6	Conclusions.....	62
Chapter 5	CMOS ISFET Sensor for Ion Imaging	65
5.1	ISFET Sensor and Ion Imaging.....	66
5.1.1	ISFET based DNA Sequencing	66
5.2	Dual-mode ISFET Sensor	67
5.2.1	Dual-mode Sensor Pixel	67
5.2.2	Dual-Mode Sensor Architecture	69

5.2.3	Dual-mode Sensor Control	70
5.3	CDS Readout with Suppressed FPN.....	71
5.3.1	CDS for Optical Sensing.....	72
5.3.2	CDS for Chemical Sensing	73
5.3.3	Global Amplifier	74
5.3.4	Pipelined ADC	76
5.4	Measurement Results	77
5.5	Conclusions.....	82
Chapter 6	CMOS Optical Sensor for Microfluidic Contact Imaging	83
6.1	CMOS Image Sensor and Contact Imaging.....	86
6.1.1	CMOS Image Sensor	86
6.1.2	High Dynamic Range Pixel	87
6.1.3	Column Parallel Readout	88
6.1.4	Super-resolution Processing.....	89
6.2	Lensless Microfluidic Cytometer.....	93
6.2.1	System Design	93
6.2.2	Cell Sample Preparation	98
6.3	Contact Image Processing	98
6.3.1	Temporal-differencing based Flowing Cell Detection	98
6.3.2	ELM-SR based Flowing Cell Recognition	99
6.3.3	Flowing Cell Counting.....	104
6.4	Measurement Results	104
6.4.1	CMOS Image Sensor Chip.....	104
6.4.2	Counting Performance Characterization	108
6.4.3	Off-line ELM-SR Reference	110

6.4.4	On-line ELM-SR Recognition and Counting	112
6.5	Conclusions.....	115
Chapter 7	Conclusions and Future Research.....	117
7.1	Conclusions.....	117
7.2	Recommendations for Further Work	118
	Author's Publication.....	119
	Bibliography	123

List of Figures

Figure 1-1 Global ageing trends for the percentage of the total population at 60-year old and over in 2012 and 2050 [1].	2
Figure 1-2 BD FACSCount™ cell cytometer system.	3
Figure 2-1 A typical microscope and its optical path with objective lens and eye piece. To reach high-resolution imaging capability, bulky, expensive and sophisticated lenses are required.	8
Figure 2-2 Basic principle of pulse-echo imaging.	10
Figure 2-3 Standard bulky ultrasound machine and block diagram including ultrasound transducer and transmission/reception electronics.	11
Figure 2-4 Two microfluidic devices: (a) A microfluidic channel attached on top of a CMOS image sensor for sample delivery [43]. (b) A microfluidic splitting device besides a one US cent [44].	14
Figure 2-5 Cross-sectional schematic of a unit cell on digital microfluidic arrays.	14
Figure 2-6 Contact imaging principle: (a) the object to be imaged is in direct contact with the image sensor pixel array, (b) the objects (cells) to be imaged are suspending in microfluidic channel that is attached on top of the image sensor.	16
Figure 2-7 UCLA, lensless ultra wide-field cell monitoring array platform based on shadow imaging (LUCAS). (a) Diagram of a microfluidic channel attached on top of a CCD image sensor. (b) The figure of a microfluidic chamber placed on a CCD sensor. (c) The shadow images of the CD4+ T-lymphocyte cells captured by the LUCAS system. Scale bar is 100μm [63].	18
Figure 2-8 Caltech, lensless on-chip Optofluidic Microscope (OFM) system. (a) OFM integrates microfluidic channel with an aperture array fabricated on top of a CMOS image sensor. (b) The chip assembled on a CMOS image sensor besides a US quarter coin. (c) The chip can be upright operated to enable gravity-driven flow to avoid the use of external pumps [59].	19
Figure 2-9 Lensless digital holographic on-chip microscope. (a) The image of the digital holographic on-chip microscope system. (b) Diagram of the system. (c–e)	

Microscope images of <i>S. pombe</i> yeast cells imaged under 10× objective lens. (f–h)	
The detected 2D orientation of the cells by the holographic microscope [56].	20
Figure 2-10 (a) Traditional ISFET fabricated by special process; (b) ISFET fabricated through standard CMOS process.	21
Figure 2-11 Concept of the optical and potential dual-mode CMOS image sensor, which combines the light sensing pixel and potential sensing pixel together [75].	23
Figure 2-12 Fabricated dual-image optical and electrochemical CMOS image sensor.	24
Figure 2-13 Schematic of the multimodal optical and pH CMOS image sensor.	25
Figure 3-1 Microfluidic channel masks designed by AutoCAD.	28
Figure 3-2 PDMS mold fabrication process.	29
Figure 3-3 Spin speed vs. thickness for selected SU-8 resists [79].	30
Figure 3-4 Microfluidic channel fabrication process using soft lithography technique.	32
Figure 3-5 Contact microfluidic imaging system model.	34
Figure 3-6 Resolution model for the lensless microfluidic imaging system, which shows the captured image intensity profile in one dimension.	35
Figure 3-7 (a) The relationship between object distance and system resolution. (b) The relationship for sensor dynamic range and pixel size.	36
Figure 4-1 Block diagram of a typical ultrasound imaging system, including the multi-channel AFE IC, CMUT array, and signal processor.	42
Figure 4-2 Overall system floor plan for 2-D multi-channel analog front-end IC.	43
Figure 4-3 Two-channel analog front-end IC unit cell consisting of two HV pulsers, two HV protection switches, and a shared low-noise preamplifier.	44
Figure 4-4 (a) Diagram of CMUT array, (b) one CMUT element, (c) one CMUT cell, (d) cross-section view of CMUT cell, (e) top view of CMUT cells.	47
Figure 4-5 Equivalent electrical model for the CMUT device in immersion.	47
Figure 4-6 Timing diagram of analog front-end operation.	47

Figure 4-7 Block diagram of transmitter front-end IC.....	49
Figure 4-8 Schematic of 1.8-to-5 V level-shifter.....	49
Figure 4-9 Schematic of 5-to-25V level-shifter and HV output driver.	50
Figure 4-10 Resistive feedback TIA schematic.	52
Figure 4-11 Simulated closed-loop frequency response.....	53
Figure 4-12 Simulated input referred current noise.....	54
Figure 4-13 Chip microphotograph of two-channel analog front-end IC.....	55
Figure 4-14 The fabricated 16×16 2D array of ultrasound analog front-end cells.	56
Figure 4-15 Measured transient response of the HV pulser.	57
Figure 4-16 Measured TIA gain and bandwidth simulation vs. measurement results..	57
Figure 4-17 TIA input referred noise measurement results.	58
Figure 4-18 Acoustic transmission testing setup for implemented AFE IC and fabricated CMUT sample.....	59
Figure 4-19 Measured hydrophone output voltage signal.	60
Figure 4-20 Acoustic pulse-echo testing setup for implemented AFE IC and fabricated CMUT sample.....	61
Figure 4-21 Measured pulse-echo response.....	62
Figure 5-1 existing large ISFET array for DNA sequencing, which shows the problem of sequencing accuracy due to pH crosstalk and VT variation.....	65
Figure 5-2 Schematic and cross-sectional pixel layout of dual-mode sensor for DNA sequencing with microbead by contact imaging and ion sensing.	68
Figure 5-3 (a) Cross-section layout of the dual-mode CMOS ISFET pixel. The existence of microbead can be detected by contact imaging, and the pH shift caused by nucleotide incorporation can be measured by ion sensing. (b) The top view of the dual- model pixel.....	69
Figure 5-4 (a) Ion-image sensor chip architecture and (b) die micrograph.	70
Figure 5-5 CDS readout schematic for dual-mode sensor.	71

Figure 5-6 CDS readout timing diagram for both (a) optical mode and (b) pH mode.	72
Figure 5-7 The global core amplifier circuit.....	75
Figure 5-8 The global amplifier AC simulation results.....	75
Figure 5-9 12-bit Pipelined ADC architecture.....	76
Figure 5-10 12-bit Pipelined ADC INL/DNL simulation results.	77
Figure 5-11 Micrograph photo of the dual-mode sensor chip and testing setup.	78
Figure 5-12 The correlated maps of distributed microbeads: (a) contact images; and (b) pH values.	79
Figure 5-13 Measurement results: (a) pH sensitivity of dual-mode ISFET sensor.	80
Figure 5-14 The comparison with commercial pH meter for bacteria (E. Coli) culture solution with glucose at different time intervals.....	80
Figure 5-15 Measurement results: spatial FFT of readout voltage variations (a) with CDS and (b) without CDS readout.	81
Figure 6-1 Different contact imaging systems without optical lens. (A) Static contact imaging system. (B) Microfluidic contact imaging system with capillary flow. (C) The proposed microfluidic contact-imaging cytometer system with continuous flow: (C1) bonding process; (C2) overall system structure.	84
Figure 6-2 System architecture of the super-resolution CMOS image sensor.....	86
Figure 6-3 3T large-sized pixel structure: (a) layout. (b) A-A' cross section.	87
Figure 6-4 (a) Schematic diagram of one column signal path for column-parallel readout. (b) Digital timing control diagram for the signal readout chain.	89
Figure 6-5 Working principle of multi-frame super-resolution processing.....	91
Figure 6-6 Working principle of the proposed single-frame SR algorithm.....	92
Figure 6-7 Microfluidic contact-imaging cytometer system for flowing cell detection, recognition and counting. (A) Cell shadow image by contact imaging. (B) Captured video of flowing cells. (C) CMOS image sensor board schematic with external controls. (D) System board of the developed microfluidic cytometer.....	94
Figure 6-8 Microfluidic channel bonding process.....	95

Figure 6-9 The relation between the PDMS thickness and spin-coating speed and time [149].	96
Figure 6-10 Structure of the extreme learning machine model.	99
Figure 6-11 ELM-SR processing flowchart. The training is performed off-line to generate a reference model that can map the interpolated LR images with the HF components from the HR images; and the testing is performed on-line to recover a SR image from the input LR image with the reference model.	102
Figure 6-12 Flowing cell recognition flowchart. The detected LR image is processed with ELM-SR to obtain SR images according to different off-line trained models. Then, the SR images are compared with typical HR cell images in the library with cell categorized to one type that has the largest MSSIM.	103
Figure 6-13 Chip photo of the taped out CMOS image sensor with GF standard CMOS 0.18 μ m process.	105
Figure 6-14 Readout timing results by the designed column-parallel CMOS image sensor.	106
Figure 6-15 Bio-microfluidic device that can separate cancer cells out from normal cells by using gravity.	107
Figure 6-16 Particle image processing results of single-frame SR. (a) particle flowing in microfluidic device. (b) Original 8 $\times$ 8 LR particle image. (c) 32 $\times$ 32 SR image processed by the on-chip single-frame SR processing.	108
Figure 6-17 Comparison of concentration measurement results for 6 μ m microbead solution between the developed microfluidic cytometer and the commercial flow cytometer. The average counting result of the developed microfluidic cytometer matched well with that of the commercial cytometer with 8% error.	108
Figure 6-18 Comparison of counting results of different microbead concentration solutions between the developed microfluidic cytometer and the commercial flow cytometer. (A) Measurement results correlate well between the developed system and the commercial one ($y=0.97x-8$, correlation coefficient=0.996). (B) The Bland-Altman analysis of the measurement results between the developed one and the commercial	

one show a mean bias of $-13.6\mu\text{L}^{-1}$, the lower 95% limit of agreement by $-61.0\mu\text{L}^{-1}$, and the upper 95% limit of agreement by $33.8\mu\text{L}^{-1}$ 109

Figure 6-19 ELM-SR off-line training images for HepG2 and RBC cells. (A) The original HR images for HepG2 cell with two different appearances; and the same for RBC cells. (B) The corresponding LR images. (C) The interpolated images of LR images, which cannot show HF details. (D) The extracted HF components. The scale bar indicates $5\mu\text{m}$ 111

Figure 6-20 ELM-SR on-line testing results for HepG2 and RBC cells. The resolution is improved by 4X after ELM-SR processing. (A) The HepG2 on-line testing image and the recovered SR image. (B) The RBC on-line testing image and the recovered SR image. (C) The comparison of MSSIM for different SR images obtained under different training models. The detected HepG2 and RBC can be correctly categorized to its type as the SR image recovered by corresponding ELM-SR model produces a larger MSSIM when compared to each cell HR library. The scale bar indicates $5\mu\text{m}$ 112

Figure 6-21 Commercial flow cytometer counting results for the mixed RBC and HepG2 cells. The absolute counts of RBC and HepG2 are 1054 and 978 with the ratio of RBC/HepG2 by $51.9\%:48.1\% = 1.08:1$ 113

List of Tables

Table 3-1 List of key parameters for contact microfluidic imaging system model.	33
Table 4-1 Key design parameters for the custom designed CMUT device.	46
Table 4-2 Summary of the measured transceiver front-end IC performance.	58
Table 5-1 Dual-mode sensor chip design specifications.....	77
Table 5-2 Comparison of state-of-art ISFET sensors.	81
Table 6-1 Summary of characteristics of the super-resolution CMOS image sensor.	106
Table 6-2 Measured RBC and HepG2 counting results of the developed microfluidic cytometer with ELM-SR based cell recognition.....	114

Chapter 1

Introduction

1.1 Motivation

The world population is rapidly ageing with proportion of people aged 60-year-old and over, growing faster than any other age groups, as shown in Figure 1-1 [1]. According to the expectation of world health organization [2], between 2000 and 2050, the world's population over 60 years will double from about 11% to 22%, the absolute number of people 60-year-old and over will also increase from 605 million to 2 billion. Among the ageing countries, the most dramatic changes are now taking places in low and middle income countries, such as Cuba, Iran, Mongolia, and China, which are usually resource limited. Along with the current aging society also come special personalized healthcare challenges. Therefore, it is imperative to prepare healthcare and diagnosis systems to meet the needs of personalized bio-imaging diagnosis of the aging populations.

Over the past several decades, biomedical imaging diagnostic techniques such as microscope imaging [3], ultrasound imaging [4-6], flow cytometry [7-9], and even optical genetic sequencing [10-12] have improved the early diagnosis, accurate monitoring of existing diseases, and understanding of the underlying causes and mechanisms of disease. However, current diagnosis systems are usually bulky, expensive, inflexible for multiple functionality diagnosis, need professional personnel to operate, and usually only available in established hospitals or clinics. These problems pose significant challenges for the healthcare of aging populations, especially in low-income developing countries. For example, FACSCount, as shown in Figure 1-2, is one commercial flow cytometer system that can provide absolute and percentage counting results of various types of cells, such as red blood cells (RBCs), leukocytes, and CD4 T-lymphocytes. Clinicians rely on this system to diagnose the stage progression of HIV/AIDS, guide the treatment decision for HIV-infected persons, and evaluate the effectiveness of Antiretroviral Therapy (ART) [13]. However, it is only available in laboratory settings due to the limitations of bulky desktop size,

prohibitive equipment costs (\$27,000), high maintenance and reagent costs (\$5–\$20), low throughput (30–50 samples/day), requiring an experienced operator, etc [14]. Thus, it cannot meet the personalized healthcare needs in the ageing society. As a result, the portable and affordable biomedical devices that miniaturize the bulky and expensive bio-instruments are required for point-of-care (POC) diagnosis [15-17].

Figure 1-1 Global ageing trends for the percentage of the total population at 60-year old and over in 2012 and 2050 [1].

Biomedical diagnosis at the POC means to perform the diagnosis by a clinician or patient without the need for a clinical laboratory. The testing results are timely, which allow rapid clinical decision-making and treatment. Due to the low-quality medical care condition, non-invasive diagnosis is also usually required for POC. The development of portable and non-invasive diagnostic and monitoring bio-instruments

for POC testing can lead to a paradigm shift from conventional curative medicine, to predictive and personalized medicine [18].

Figure 1-2 BD FACSCount™ cell cytometer system.

For bio-instrument miniaturization and the realization of POC diagnostic tools, portable, low cost, low sample and power consuming, fast responding, and robust systems that are easy to use and maintain are demanded. One primary assistance comes from the development of standard complimentary metal-oxide semiconductor (CMOS) fabrication technology, which allows people to create low-cost and miniaturized CMOS integrated sensors from multimodal domains with on-chip processing capability that can be mass-produced easily. In addition, the recent advancement of lab-on-a-chip (LOC) technology [19-23] has provided a promising integration platform solution of microfluidics [16], microelectromechanical systems (MEMS), electronics, and sensors [24-27], which allows a sensing function through a near-field contact imaging. As such, there are no optical lens and other bulky mechanical components.

However, along with the requirements and opportunities of building LOC platforms, there is also a challenge to develop CMOS multimodal sensors for system integration with MEMS and microfluidic channels from device and circuit perspective.

Meanwhile, there is also a challenge to fuse and recover data from multimodal domains that can help provide high accuracy and throughput measurement from system application perspective.

1.2 Research Objective and Major Contributions

Towards the personalized bio-imaging diagnosis for aging society, miniaturized bio-instrument with non-invasive sensing capability is demanded. As such, the conventional bulky optical lens and other mechanical components can no longer be applied. One needs to develop LOC integrated systems that integrate microfluidics, MEMS and CMOS sensors for bio-imaging diagnosis. The challenge of such an integrated system comes to the need of multimodal contact imaging. Therefore, in this thesis, we have particularly studied the CMOS multimodal sensor for LOC integrated bio-imaging diagnosis system, including: 1) CMOS (capacitive-micromachined-ultrasonic-transducer) CMUT sensor for non-invasive ultrasound imaging towards the glaucoma diagnosis; 2) CMOS (ion-sensitive-field-effect-transistor) ISFET sensor for ion imaging towards the DNA sequencing application; and 3) CMOS optical sensor for microfluidic contact imaging towards the cell detection, recognition, and counting application. We will illustrate the need and application of the three corresponding bio-imaging diagnosis methods as well as design problems addressed when being miniaturized, which can be summarized as follows.

- Firstly, we illustrate one device-level design work using the example of ultrasound imaging. A two-channel analog front-end (AFE) IC for interfacing multi-channel high frequency CMUT array is developed with three-dimensional high resolution imaging capability for glaucoma diagnosis. The main challenge is the process integration between MEMS array and CMOS readout circuit, where flip-chip bonding is deployed. With the use of 30V high-voltage 0.18 μ m Bipolar-CMOS-DMOS (BCD) technology, the proposed AFE IC cell is designed to consist of two high-voltage (HV) pulsers in the transmit path, and a shared single low-noise pre-amplifier in the receiver path for area reduction. The electrical functionality of the proposed AFE IC is characterized in which the HV pulser generates a delay of 16.2ns between the 33ns input trigger pulse and the HV output pulse while driving the load capacitance of 43pF from 0 to 30V. And the

low-noise preamplifier achieves over 60dB Ω transimpedance gain with 27.5pA/sqrt(Hz) input referred noise current at 35MHz. A successful pulse-echo acoustic testing is also demonstrated with the developed AFE IC that integrates the CMUT sample in an oil-immersed environment.

- Secondly, we discuss one circuit-level design work using the example of ion imaging. A 64 $\times$ 64 1200fps dual-mode CMOS ion-image sensor is demonstrated with suppressed fixed-pattern-noise (FPN) for accurate high-throughput DNA sequencing. The main challenge of the traditional ISFET-based ion imaging is lack of faulty pH value detection. In this work, we show the solution by pruning sensed data with reference from multi-domain. A dual-mode mode ISFET sensor is developed, including pH sensing from chemical domain as well as image sensing from optical domain. An ISFET with standard 4T-CMOS image sensor (CIS) pixel structure is proposed and fabricated in standard 0.18 μ m 1P6M CIS process. After addressing physical locations of DNA slices determined by the optical contact imaging, local pH value of one DNA slice can be mapped to its physical address with the accurate correlation, which can significantly improve the DNA sequencing accuracy. Moreover, pixel-to-pixel ISFET threshold voltage mismatch or FPN is reduced by a correlated double sampling (CDS) readout circuit structure that supports both image and pH modes for large-array and high-throughput application. Measurement results show a sensitivity of 103.8mV/pH and FPN reduction from 4% to 0.3% with a readout speed of 1200fps.
- Lastly, we present one system-level design work using the example of microfluidic contact imaging. A microfluidic contact imaging system has been developed with poly-dimethylsiloxane (PDMS) microfluidic channel integrated on top of CMOS image sensor for flowing cell detection, recognition and counting. The main challenge of such a lensless microfluidic system is how to improve resolution because of no optical lens. To resolve the raw spatial resolution limitation from pixel size, an extreme-learning-machine based single-frame super-resolution processing (ELM-SR) is proposed that can recover high-frequency loss in detected cell contacting images such that flowing cells can be still distinguished for counting. The

prototyped lensless microfluidic system obtains less than 8% counting error for absolute number of microbeads; and 0.10 coefficient of variation for cell-ratio measurement of mixed RBC and HepG2 cells in solution.

In this thesis, we have shown a thorough study to explore multimodal CMOS sensors in LOC towards the portable personalized bio-imaging diagnosis system, which could pave the way towards a variety of personalized diagnosis applications such as: 1) CMOS ultrasound sensor for non-invasive human body scanning; 2) CMOS dual-mode ion sensor for portable DNA sequencing; and 3) CMOS contact imaging sensor for point-of-care blood cell tests. Note that the primary novelty of this thesis is the design of CMOS ISFET ion-image sensor (published in IEEE Symposium on VLSI Circuits 2014). As a conclusion, the CMOS multimodal sensor based LOC system has been shown with the great potential to provide the future personalized e-healthcare solution for the coming aging society.

1.3 Organization of the Thesis

The rest of the thesis is organized as follows. Chapter 2 reviews the background of traditional bio-imaging, LOC technology, and CMOS multimodal sensors with identified challenges and opportunities. Chapter 3 discusses the fundamentals of microfluidic fabrication and contact imaging system model for LOC system. Chapter 4 to 6 further provide in-depth discussions for the CMOS CMUT sensor for ultrasound imaging, CMOS ISFET sensor for ion imaging, and CMOS optical sensor for microfluidic contact imaging, from device, circuit and system point of view, respectively. The conclusion is drawn in Chapter 7 with suggested future research.

Chapter 2

Background and Literature Review

2.1 Traditional Bio-Imaging

Over the centuries, many biomedical imaging modalities have been invented and applied for the disease diagnosis and healthcare, such as optical microscope [3], ultrasound imaging [28, 29], X-ray [30], Computed Tomography (CT) [31], Magnetic resonance imaging (MRI) [32], THz imaging [33], etc. In the following, two of the most widely used traditional bio-imaging modalities for personalized diagnosis, namely high-resolution optical microscope imaging and non-invasive ultrasound imaging, are discussed.

2.1.1 High-resolution Optical Microscope Imaging

Microscope is an optical instrument that produces a magnified image of an object under inspection compared with what the naked human eye could observe using visible light and a system of lenses. Since invented more than 400 years ago by two Dutch spectacle makers Hans and Zaccharias Janssen, then improved by Galileo and Antonie van Leeuwenhoek, the optical microscope is the leading high-resolution visualization tool and the gold standard for biomedical imaging at cell-level [34].

To achieve micrometer or sub-micrometer resolution, almost all microscopes require precise and expensive optical lenses, as well as a large distance between the object lens and eyepiece lens for the light to travel and reshape, as the optical path shown in Figure 2-1. The object to be seen is illuminated by a light source. As light passes through the object, the objective lens (i.e., lens closest to the object), produces a magnified object image in the primary image angle. The eyepiece (i.e., lens that people look into) acts as a magnifier that produces an enlarged image of the image generated by the objective lens. The overall magnification of the microscope system is the multiplication of the magnification of both objective and eyepiece. The principle of magnification is based on the thin lens approximation as follows,

$$\begin{cases} \frac{1}{L_i} + \frac{1}{L_o} = \frac{1}{F} \\ M = \frac{L_i}{L_o} = \frac{H_2}{H_1} \end{cases} \quad (2-1)$$

where L_i are L_o are the image distance and object distance, F is the focal length of the objective lens, M is the magnification factor of the objective lens, H_1 and H_2 are the size of object and image. Therefore, a significant space L_i is usually required to produce a large microscope magnification, which is one main difficulty for the optical microscope minimization.

Figure 2-1 A typical microscope and its optical path with objective lens and eye piece. To reach high-resolution imaging capability, bulky, expensive and sophisticated lenses are required.

Compared with the earliest compound microscope, the current design has evolved to incorporate multiple lenses, filters, polarizers, beamsplitters, sensors, illumination

sources, and a host of other components, etc., aiming to improve resolution and sample contrast. However, this basic microscope design has undergone very few fundamental changes over the centuries, which makes it bulky, expensive, and sophisticated, hence not suitable for POC diagnosis.

2.1.2 Non-invasive Ultrasound Imaging

The high-resolution optical microscope for cell-level diagnosis usually deals with the human tissue or blood samples, which need invasive procedure to obtain. An invasive procedure means to penetrate or break the skin or enter a body cavity. For centuries, invasive procedures such as catheterization and exploratory surgery are routinely performed to patients who are critically ill or to diagnose their sources of illness. Even until now, many therapeutic methods still require invasive surgery, which is dire, painful, high-cost, and requires long time to recover. However, after the discovery of the X-Ray by a German physicist, W. C. Roentgen, non-invasive diagnostic bio-imaging was first performed in 1895 [30]. For the first time, physicians can see inside the body without the need to perform invasive surgery. After that, diagnostic bio-imaging becomes a non-invasive way to observe internal structures and organs.

The non-invasive diagnostic bio-imaging techniques not only include X-ray, but also ultrasound imaging. The ultrasound imaging is an imaging technique which provides a two-dimensional, cross-sectional reflection image of the scanned object using pulse-echo (backscattering) response of an ultrasound wave. Compared to X-ray, the non-invasive ultrasound imaging [4, 5, 35] has much less harmful characteristics to the human body as it is a non-ionizing radiation. Moreover, ultrasound imaging also has the advantages of lower cost and real-time imaging capability compared with MRI [32] and CT [31], making it a suitable option for diagnostic instrument miniaturization.

The commonly used brightness mode ultrasound imaging is based on the pulse-echo response of an ultrasound wave, which generates a two-dimensional cross-sectional reflection image of the scanned object. At first, a transducer (or transducer array) generates an ultrasound pulse in response to an electrical excitation pulse. Then the pulse propagates through the medium of interest. After certain attenuation and absorption, some of the reflections return to the transducer and reconverted to electrical signals. The amplitude information of the detected signals will be converted

to brightness information of the target object, resulting in the detected ultrasound image. This basic principle of pulse-echo imaging is shown in Figure 2-2.

Figure 2-2 Basic principle of pulse-echo imaging [36].

Typically, medical applications utilize ultrasound frequencies between 1 and 50 MHz. For instance, the low frequencies between 1 and 10 MHz can be applied to penetrate tissues to a depth of 5 to 20 cm and still return signals of sufficient strength to form a diagnostic image. Higher-frequency ranges between 20 and 50 MHz provide ultrasound imaging of smaller organs such as the gastrointestinal tract, sections of skin, and intravascular structures. Therefore, for applications such as glaucoma diagnosis for the aging people, higher-frequency range are usually required [29].

A typical commercial ultrasound diagnostic machine usually includes a transducer probe that transmits and receives acoustic waves, a computer that processes the signals, a screen to display the images, a transducer control panel for adjusting the sound waves, and others interfaces such as a keyboard, a printer, and hard disk storage, as shown in Figure 2-3. The cable connection between the transducer device and the TX/RX block is usually of high cost. The transducers are usually at high voltage, which can even go up to 100V, and complicated front-end electronics composed of

discrete IC chips of pulzers, preamplifiers, time gain control (TGC), analog-to-digital converter (ADC), etc., are used. Hence, this system is also bulky, expensive, and need a trained professional to operate, which limits its usage in resource poor settings. Portable ultrasound imaging systems become highly demanded in POC medical services.

Figure 2-3 Standard bulky ultrasound machine and block diagram including ultrasound transducer and transmission/reception electronics.

2.1.3 Problems of Traditional Bio-imaging

The above discussions introduce two most widely used bio-imaging modalities for healthcare, i.e., the optical microscope imaging and the ultrasound imaging. For the future POC personalized bio-imaging diagnosis application, it is obvious that the bulky and expensive systems need to be miniaturized. There are three main challenges here:

- 1) From basic imaging principle, the traditional bulky lens based optical imaging principle needs to be changed to a more compact and portable imaging method;
- 2) From device and circuit level, the complicated integration of discrete bio-imaging electronic circuits as well as the further integration with MEMS devices such as transducer need to be on-chip integrated;

- 3) From system level, multiple imaging modalities need to be integrated on one sensor with further data fusing and recovering from multimodal domains to enable high accuracy as well as more powerful multiple diagnostic functions.

To solve these problems, the recently developed lab-on-a-chip (LOC) technology provides a promising integration platform solution, which can integrate microfluidics, MEMS, and CMOS sensors all together to create an on-chip imaging system [37-41]. The lab-on-a-chip bio-imaging is discussed in the following section.

2.2 Lab-on-a-chip Bio-imaging

2.2.1 Lab-on-a-Chip with Microfluidics

As is implied by the name, the lab-on-a-chip means a minimized chip-scale (a few square centimeters in size) device that has the ability to perform one or several laboratory functions [37-41]. To tackle the burden of personalized heal-care in a global era with continuously growing population and inefficient distribution of resources, the effective solution can only come from the technologies that have high productivity. And the LOC system can suitably combine the advantages from the high productivity CMOS fabrication technology and microfluidics technology [16, 38, 42] to tackle the above mentioned challenges.

Firstly, in LOC system, the optical imaging can utilize the so-called *Contact Imaging* principle [43], which removes the usage of the intermediate projection lens by placing the object to be imaged in close proximity (or in contact) with a image detector, which is usually a Charge-Coupled Device (CCD) or CMOS image sensor.

Secondly, the discrete electronic circuits and transducer integration challenge can also be resolved with CMOS integrated circuit design that includes the functions of all discrete circuit components as well as integration with MEMS devices on-chip.

Moreover, in this on-chip contact sensing configuration, the functionality of image sensor can also be extended to other sensing technologies for various measurement targets, such as electrochemical, electric, and chemical (pH) imaging, which is quite useful especially for biological and biochemical sample analysis. Then, the CMOS fabrication technology can make it possible to implement both optical imaging and electric/chemical sensing functionalities in one on-chip CMOS image sensor, thus multiple imaging modalities are integrated together. By further fusing and recovering

data from multimodal domains, it can help provide high accuracy measurement from system application perspective.

Since the LOCs usually deal with chemical or small object analysis such as cells or microorganisms in extremely small fluid volumes (micro liters or even pico liters), it is closely related to the microfluidics [16, 38, 42] for sample preparation, delivery, and processing on-chip. The reduced sample volume, portability, low cost, and the possibility to integrate new analytical device or CMOS electronics are some of the key advantages over the traditional laboratory-scale testing. Next, more details about the microfluidics and contact imaging for LOC systems are introduced.

2.2.1.1 Microfluidic Fundamentals

Microfluidics is a multidisciplinary engineering field where chemistry, physics, engineering and biotechnology intersect to develop microscale chips where the fluid analysis can take place. The control and manipulation of the micro fluids are usually precisely and geometrically constrained to a microscale or nanoscale volume. The flow of a microfluid through a microfluidic channel can be characterized using the Reynolds number as follows:

$$Re = \frac{LV_{avg}\rho}{\mu}, \quad (2-2)$$

where L is the most relevant channel length scale, V_{avg} is the average flow velocity, ρ is the fluid density, and μ is the fluid viscosity. For many microchannels, $L=4A/P$ where A is the channel cross sectional area, and P is the wetted channel perimeter. Re is usually much less than 100 (often less than 1.0) due to the small dimensions of microchannels. In this Re regime, microfluid flow is completely laminar without turbulence. Thus the micro sample can be transported in a relatively predictable manner through microchannels. When the Re increases to the range of 2000, the micro flow starts to change to turbulent flow. Figure 2-4 shows two microfluidic devices. The left one is a microfluidic channel attached on top of a CMOS image sensor for sample delivery. The right one is a microfluidic splitting device next to a one US cent piece. Both of them clearly show the tiny dimension of microfluidics.

There are usually two methods of fluid actuation, namely electrokinetic flow and pressure driven flow. In electrokinetic flow, the fluids are driven by electroosmotic pumping. While in pressure driven flow, the fluid is driven through the channel by positive displacement pumps such as syringe pumps. The advantage of electrokinetic flow over pressure driven flow is that it can couple other on-chip electronic applications. However, electrokinetic flow often requires very high voltages. Without power supplies off-chip, it is difficult to be miniaturized. One other significant disadvantage of electrokinetic flow is the variability in surface properties. For example, the proteins absorbed to the channel walls can substantially change the surface charge characteristics, and hence lead to the change of fluid velocity. This problem will result in unpredictable and irregular long-term time dependency in the microfluid flow.

Figure 2-4 Two microfluidic devices: (a) A microfluidic channel attached on top of a CMOS image sensor for sample delivery [44]. (b) A microfluidic splitting device besides a one US cent [45].

Figure 2-5 Cross-sectional schematic of a unit cell on digital microfluidic arrays.

There is also another kind of microfluidics, the droplet-based digital microfluidics as shown in Figure 2-5, which employs electrowetting-on-dielectric to precisely manipulate discrete droplets of biochemical samples and reagents at microliter or picoliter volumes under digital clock control [46-54]. It can combine electronics with biology, and integrate together a number of bioassay operations, such as sample preparation, separation, mixture, analysis, and detection [49, 50, 52, 53] to form a miniaturized digital microfluidic biochip (DMFB) and automate laboratory procedures in immunoassays, biochemistry and molecular biology [47]. Compared to conventional expensive and cumbersome laboratory procedures, DMFBs show the advantages of lower cost, higher sensitivity, easier system integration, and less likelihood of human errors [46-54]. In contrast to continuous-flow microfluidics, digital microfluidics works much the same way as traditional bench-top protocols, only with much smaller volumes and much higher automation. Therefore, a wide range of established chemistries and protocols can be seamlessly transferred to a nanoliter droplet format.

2.2.1.2 Microfluidic Advantages

There are several advantages of using microfluidic devices in clinically useful technologies and perform biomedical imaging diagnosis:

- 1) Microfluidic technologies make it possible to fabricate highly integrated devices so that several different functions, such as blood or saliva filtering, desalting, denaturation, concentration and derivatization, can be performed on the same chip.
- 2) Small volumes lead to fast analyses; moreover, the small amount of reagents and analytes used is especially important for expensive reagents.
- 3) Material cost is negligible in micro-systems. The techniques used to fabricate microfluidic devices are relatively inexpensive and suitable for highly multiplexed devices and mass production.

After the fundamentals of microfluid flow and sample transportation are well developed, the microfluidics is recently developing towards the integrated devices that incorporate multiple fluidic, mechanical, and electronic components as well as various chemical processes onto a single chip. It has been a major push towards the bio-imaging instrument miniaturization.

2.2.2 Contact Imaging

2.2.2.1 Contact Imaging Principle

Conventional optical microscope imaging systems require intermediate bulky lens for magnification, which usually constrains the size, weight, and cost of such systems, and hence leads to the difficulty of miniaturization for POC applications. One promising solution is the so-called *Contact Imaging*, which directly couples the image detection array (i.e. image sensor array) with the sample of interest. Thus, the sample image can be captured by directly projecting light through it, or by detecting the light emitted from the object. This principle is shown in Figure 2-6. Note that the object can also be placed in microfluidic channel that is directly integrated on top of the image sensor.

Figure 2-6 Contact imaging principle: (a) the object to be imaged is in direct contact with the image sensor pixel array, (b) the objects (cells) to be imaged are suspending in microfluidic channel that is attached on top of the image sensor.

Contact imaging has significant advantages over conventional lens based imaging in light collection efficiency because of its short object distance to the sensor array. No loss is caused by optics here. Moreover, contact imaging systems have different geometrical constraints over spatial resolution compared with lens based imaging. In conventional optical imaging systems, the image resolution is determined by the number of pixels in the photo detect array as the scene is entirely projected to the sensor array by optics. By increasing the number of pixels, the spatial resolution for conventional imaging is increased. Differently in contact imaging, as the image is directly projected from the object to the sensor array, the resolution is determined by the pixel dimensions. And only by reducing the pixel size can we improve the spatial resolution for contact imaging [43].

As discussed before, in contact imaging system, the microfluidic channels are usually used to deliver the samples of interest. Due to these differences and advantages over conventional lens based imaging, contact imaging has been previously studied for various applications such as in vitro and in vivo biomedical imaging, as well as LOC systems.

2.2.2.2 Contact Imaging Systems

For POC diagnosis, the CIS is applied to miniaturize the on-chip biomedical imaging systems through the principle of contact imaging. With microfluidics for sample delivery/capture and lensless optical sensing and detection, contact imaging systems can be applied to ultra wide-field cell monitoring array [55-57], digital in-line holography [57-59], optofluidic microscopy [60, 61], and lensless on-chip microscopy [44, 62, 63].

There are generally three basic types of on-chip contact imaging methods, namely the Optofluidic Microscopy (OFM) [60, 61], the lensless ultra wide-field cell monitoring array platform based on shadow imaging (LUCAS) [55, 56, 62], and the lensless digital holography system [57].

The first method is LUCAS, which can detect and count cells on-chip [62] by recording and analyzing their shadow images. The cells are located between two microscope slides or in a microfluidic channel that is attached on the CCD/CMOS image sensor. And they are uniformly illuminated by an incoherent white light source or a laser beam. During cell counting, the cell shadow image pattern is recorded by a

CCD/CMOS sensor. The advantage of this system is the high-speed counting of statically captured cells. But the main problem for is low resolution.

Figure 2-7 UCLA, lensless ultra wide-field cell monitoring array platform based on shadow imaging (LUCAS). (a) Diagram of a microfluidic channel attached on top of a CCD image sensor. (b) The figure of a microfluidic chamber placed on a CCD sensor. (c) The shadow images of the CD4⁺ T-lymphocyte cells captured by the LUCAS system. Scale bar is 100µm [64].

The second Optofluidic Microscopy (OFM) [60, 61] integrates a microfluidic channel on top of a CMOS image sensor. During imaging, the samples of interest are flowing through the microfluidic channel with illumination from a Light Emitting Diode (LED) light source from above. Then the sample shadow images are directly captured by the CMOS image sensor below. To improve resolution, masks of nanometer-sized aperture array are fabricated above the CMOS pixel array, so the target object images can be scanned by the sensor when flowing across the aperture array as shown in Figure 2-8. The OFM system can work in an upright position. By utilizing the gravity-driven flow, the need for external pumps can be eliminated. However, this is only suitable for elongated samples such as *Caenorhabditis elegans* or round worm. If spherical or ellipsoidal samples such as cells or bacteria are tested, external electrokinetic pump should be used. The OFM provides a minimized microscope solution compared with conventional lens-based optical microscope. But

the challenge for such OFM to work efficiently is that the flow rate should be constant and the sample orientation should not be greatly changes. These problems also limit its POC applications.

Figure 2-8 Caltech, lensless on-chip Optofluidic Microscope (OFM) system. (a) OFM integrates microfluidic channel with an aperture array fabricated on top of a CMOS image sensor. (b) The chip assembled on a CMOS image sensor besides a US quarter coin. (c) The chip can be upright operated to enable gravity-driven flow to avoid the use of external pumps [60].

The third type is the digital holography [65], which measures one object's interferometry scattered light and computationally generate the images of target objects, as shown in Figure 2-9. The samples are illuminated with a spherical wavefront generated by focusing a laser beam through a pinhole. With the focused light as reference beam and scattered light as object beam, the CMOS image sensor underneath captures the interference pattern caused by the superposition of these two

wavefronts, i.e., the hologram. Compared to the conventional microscope, which only has 2D focal plane, digital holography can record the 3D image for the object.

Both OFM and digital inline holography methods achieve a spatial resolution smaller than the physical sensor pixel size. However, digital inline holography works well with samples statically prepared on glass slides, yet the OFM works with microfluidic channels for sample delivery.

Figure 2-9 Lensless digital holographic on-chip microscope. (a) The image of the digital holographic on-chip microscope system. (b) Diagram of the system. (c–e) Microscope images of *S. pombe* yeast cells imaged under 10× objective lens. (f–h) The detected 2D orientation of the cells by the holographic microscope [57].

To attain higher resolution images, Bishara et al. combined the inline holography with a multi-frame super-resolution approach to effectively improve the image spatial resolution [66]. The multi-frame super-resolution approach is a method that combines information from multiple sub-pixel-shifted low resolution (LR) images to create one single high resolution (HR) image. The principle of super-resolution is over-sampling

in the time domain. Actually, the super-solution algorithm can also be implemented in the OFM. The advantages for this are that the microscopy scheme can be greatly simplified and the aperture array can be replaced, while still keeping the high resolution. The sub-pixel resolving optofluidic microscope (SROFM) is based on this concept [44, 63]. The developments of microscope on-chip pave the way for the cost-effective bio-imaging application for the POC diagnosis in resource-limited settings.

2.2.3 Chemical Imaging

With the on-chip contact sensing configuration, the functionality of sensor can be extended to sensing technologies such as electrochemical, electric, and chemical imaging. Sensors that are capable of biochemical analysis have aroused particular interest for biomedical diagnosis. One useful parameter in biochemistry is the hydrogen ion (H^+) concentration, or pH. And it is usually needed to measure the spatiotemporal profile of pH in certain areas and to show the image result of the distribution, such as in cell culture, the monitoring of acidification in cells can indicate their metabolic activity [67]. Another recent application is the DNA sequencing system (Ion Torrent [68]) utilizing CMOS ISFET sensors. Such chemical imaging can be accomplished with the use of ion-sensitive field-effect transistor (ISFET) based sensor array.

Figure 2-10 (a) Traditional ISFET fabricated by special process; (b) ISFET fabricated through standard CMOS process.

Traditional ISFET device [69] is shown in Figure 2-10(a). The gate-oxide is

designed with ion-sensitive membrane, which is expensive and not compatible with CMOS process. As in Figure 2-10(b), Bausells first integrated ISFET in 1 μ m and 1-poly 2-metal standard CMOS process with a poly-gate connected to the top metal and passivation layer of Si₃N₄ [70]. As Si₃N₄ is observed to have good response to ion density change with a sensitivity of 45–56 mV/pH [71], the V_T of ISEFT device thereby correlates to the solution pH. Thus, an array of ISFETs can be employed to form a chemical imager, which generates a spatiotemporal pH map of chemical changes occurring at the surface of the CMOS die.

2.3 CMOS Multimodal Sensor

For the previously discussed optical microscope imaging, non-invasive ultrasound imaging, and chemical imaging, obviously the main component is the CMOS sensor to realize the corresponding imaging capability. In the following, these CMOS sensors for various sensing modalities are discussed.

2.3.1 CMOS Image Sensor

A CMOS image sensor is one device that comprises a two-dimensional pixel array to convert the incident light at its surface into an array of electrical signals [72-74]. These electrical signals are then read out of the image sensor under the control of column and row decoder and driver and digitized by an ADC finally to generate a digital image.

Recently, the CMOS image sensor has been widely applied in LOC bio-imaging systems such as OFM [75], SROFM [44], LUCAS [55]. After integration with microfluidics, the conventional lens-based optical microscope can be replaced. Such system has been used for portable high-resolution cell imaging and cell counting applications, as discussed in Section 2.2.2.2. The pixel size is playing a decisive role in cell detection system based on the contact imaging principle. The CMOS image sensor used in most of these LOC imaging systems are commercial sensors, such as MT9T031 in SROFM and MT9M001 in OFM. Commercial sensors are usually targeting for low power consumption electronics at a frame rate about 30fps to 60fps, and most of them do not have on-chip processing capability. Thus, the current LOC imaging systems apply off-chip imaging processing algorithm to improve the system resolution. Therefore, custom designed CMOS image sensor can incorporate

intelligent on-chip processing to integrate processes and more diagnostics from bench to bedside. It also has the advantage to integrate with other imaging modalities such as the chemical imaging, to perform multi-modal sensing.

2.3.2 Multimodal CMOS Image Sensor

In biomedical testing, some properties of a reaction or biological material can be detected optically but not chemically, and vice versa for other properties. In some cases, it is only when these properties are known together that one can draw conclusions about the state of the reaction or biological material. The field of sensor fusion has received much attention recently as the combining of sensors can improve sensing capability. Thus, multi-modal CMOS image sensors are required.

Figure 2-11 Concept of the optical and potential dual-mode CMOS image sensor, which combines the light sensing pixel and potential sensing pixel together [76].

Light and electricity are two important media used in the measurement or control of biological samples such as proteins, neural cells, and DNA. Conventionally, optical imaging is based on the microscopy technology, and electrical sensing of neural activity is through micro-electrode technology. In [76], a CMOS image sensor which can simultaneously capture optical and potential dual-mode image is demonstrated targeting on-chip applications such as DNA (or protein) microarray analysis and neural imaging. The concept of the optical and potential dual-mode CMOS sensor is shown in Figure 2-11. The sensor can work in either a high-resolution potential imaging mode (1.6mV) or a wide-range potential imaging mode (>5V) under the control of a specific operating sequence and off-chip configuration [76]. The profile of

a potential spot smaller than $50\text{ }\mu\text{m}$ is achieved. However, it is just simply integrate two different types of pixel together, i.e., interleave 88×144 light sensing pixel and 88×144 potential sensing pixel together to form a 176×144 pixel array, so the actual spatial resolution is reduced to half.

Figure 2-12 Fabricated dual-image optical and electrochemical CMOS image sensor.

In [77], a CMOS image sensor that integrates an 8×8 pixel arrayed electrochemical sensor with a 128×128 pixel APS is designed and fabricated targeting biomolecular microarray technology application, as shown in Figure 2-12. Eight column electrochemical sensing pixels are connected with eight column amplifier using current-sensing voltage followers, functioning as an on-chip potentiostats. Hence the sensor can measure 2D-arrayed voltammetry in on-chip configuration. The functions of optical and electrochemical imaging can be operated either simultaneously or independently. The range of the electrochemical current measurement was measured from about 10 nA to $100\text{ }\mu\text{A}$. Moreover, the authors expect to detect electrochemiluminescence in combination.

In [78], a multimodal image sensor for pH and light sensing in real time is proposed. Different from the previous two multimodal sensors [76, 77], in the proposed device, a photo sensor and a pH sensor are fused in the same pixel as shown in Figure 2-13,

enabling the optical and pH signals to be detected simultaneously in the same sensing area. As the pH sensing and optical sensing are built into the same area, much more accurate chemical information could be extracted without loss of spatial resolution. However, this sensor has no noise removal technique, which will lead to a pH sensing with large noise, preventing its practical usage.

Figure 2-13 Schematic of the multimodal optical and pH CMOS image sensor.

The fusion of various types of sensors has been receiving more attention recently aiming to achieve high performance in the detection of signals from multiple domains. With the challenges to develop CMOS multimodal sensors for LOC system integration with MEMS and microfluidic channels for personalized bio-imaging diagnosis, we will discuss our CMOS multimodal sensor designs in the following sections.

Chapter 3

LOC Fundamentals – Microfluidic Fabrication and Imaging Modeling

3.1 Microfluidics Fabrication

Microfluidic systems provide a powerful platform solution for lab-on-a-chip miniaturization of bio-systems. To fabricate microfluidic channels, the polymers, instead of silicon and glass, are usually used. Specifically, the poly-dimethylsiloxane, PDMS is widely used as it is inexpensive, flexible, optically transparent, and biological compatible. In this chapter, we briefly introduce the fabrication of microfluidic channels using the conventional soft-lithography [79] process.

3.1.1 Photolithography Mask

Firstly, the channel microstructures or features are designed in a computer-aided design (CAD) program, such as the AutoCAD (Autodesk, San Rafael, CA). Using commercial services, the CAD-generated patterns are printed on a transparent photolithography mask. The photolithography mask is an opaque film or plate template with transparent areas that allow light to shine through in the designed pattern. The feature size (or the lateral resolution) of the mask is determined by its material. And three types of base material are usually used to make photolithography masks, namely Quartz, Soda Lime (SL), and polyester film. Quartz and SL mask are high resolution, easy to clean, stable, but expensive. Polyester film masks have less resolution, but are low cost and much easier to handle. We use the polyester film in our microfluidic channel fabrication since we do not require high resolution below 1 μ m feature. Some examples of the designed microfluidic channel masks are shown in Figure 3-1.

Figure 3-1 Microfluidic channel masks designed by AutoCAD.

3.1.2 SU-8 Mold

After the turnaround time, the designed masks are sent back for microfluidic channel mold fabrication. Here, the negative photoresist SU-8 (SU-8 25, Microchem, MA) is used, which is a high contrast and epoxy based photoresist for micromachining. The SU-8 has superb chemical and temperature resistance and can build a thickness from 1 to 200 μ m with single spin coat process. A normal process is shown in Figure 3-2 [80].

Figure 3-2 PDMS mold fabrication process.

3.1.2.1 Substrate Pretreat.

Both silicon wafer and glass slide can be used as the SU-8 substrate. We take the glass slide as an example due to its low cost. To obtain the maximum process reliability, substrates needs to be clean and dry before applying the SU-8 resist. It is pretreated in the following way:

- 1) Soak and wash the glass slide with acetone by 1 hour at least for solvent cleaning.
- 2) Use distilled water to rinse the glass slide, and then use a stream of air or nitrogen to blow to dry.
- 3) To dehydrate the surface, bake the cleaned glass slide at 200 °C for 5 minutes on a hotplate.
- 4) Plasma treating for 10mins to further clean and dehydrate the glass slide surface.

3.1.2.2 Spin Coat

Next, the SU-8 is spin-coated using a spin machine (SCS G3P-8, Indianapolis, IN) on the clean glass slide. The spin speed directly determines the height of the mold, which is also the height of the final PDMS microfluidic channel. The relation between the spin speed and film thickness is shown in Figure 3-3. Considering that our microfluidic channel is used for normal cell flowing, we choose SU-8 25 so that the fabricated channel height can fit for normal cells such blood cell and tumor cells. The detailed steps are as follows:

- 5) Pour SU-8 onto the glass slide (about 10ml – 15ml per glass slide for static dispense), then degas in a vacuum oven for 1 hour to remove air bubble.
- 6) Spin coating for thin film deposition, the spin speed need to be set corresponding to different film heights.

Figure 3-3 Spin speed vs. thickness for selected SU-8 resists [80].

3.1.2.3 Soft Bake

After the photoresist are spin coated onto the glass slide substrate, it should be soft baked on a hot plate to evaporate the solvent as well as densify the film. As the solvent evaporation rate is affected by the heat transfer and ventilation rate, bake times should be optimized for proximity and convection oven bake process. The process is as follows:

- 7) Bake on 65°C hot plate for 5mins then 95°C for 15mins.

3.1.2.4 UV Exposure

Then, the soft baked glass slide with SU-8 film is going to do UV exposure. SU-8 is practically transparent and insensitive above 400nm. But it has high actinic absorption below 350nm. The exposure dose also affects the film thickness. And, the process is:

- 8) UV exposure of 200mJ/cm² energy for 30 seconds.

3.1.2.5 Post Expose Bake

Following the exposure, post expose bake (PEB) needs to be performed to selectively cross-link the exposed portions of the film. This is realized through a two-step contact hot plate bake process, as indicated below:

- 9) Post UV exposure bake on a 65°C hot plate for 1min then 95°C for 4mins.

3.1.2.6 Develop

After PEB, the SU-8 is going into develop using SU-8 developer. The approximate develop time is 6 minutes but it can vary widely as a function of temperature or agitation rate, i.e.,

- 10) Develop the post expose baked glass slide using SU-8 developer for 6 minutes.

3.1.2.7 Rinse and Dry

Following the development, the substrate should be rinsed using isopropyl alcohol (IPA), and then dried with a gentle stream of air or nitrogen. It then goes to the final hard bake. The detailed steps are as follows:

- 11) Rinse the substrate using IPA and dry.
- 12) Bake the substrate on a 200°C hot plate for 10mins.
- 13) Cool the slides and put them into the culture dish.

3.1.3 PDMS Replica

After the SU-8 mold is ready, we can cast the PDMS replica, i.e., microfluidic channel. The steps are as follows:

Figure 3-4 Microfluidic channel fabrication process using soft lithography technique.

- 1) Prepare the PDMS mixture for casting, a volumetric ratio of 10:1 mixture of PDMS (Sylgard 184, Dow Corning, MI) and curing agent, and pour onto the SU-8 mold.
- 2) Degas for 1hour in a vacuum oven to remove bubbles in the PDMS mixture.
- 3) Take out the glass slide and bake in oven for 2hours.
- 4) After the PDMS replica becomes dry and hard, take out and prepare to cut the channel out and peel off from glass substrate.

- 5) Use a puncher to punch holes from top of the PDMS replica for channel inlet and outlet, which are to be connected with silastic laboratory tubings to syringe pump and waste bin.
- 6) Use IPA and DI water to resin the PDMS channel. Dry the channel for the final bonding with CMOS sensors.

Now, the PDMS microfluidic channel fabrication process is completed. The whole mask, mold, and PDMS channel fabrication process flow is shown in Figure 3-4.

3.2 LOC Imaging System Model

For the contact microfluidic imaging system, the spatial resolution is determined by the pixel size and also the distance from sample to the sensor plane. Thus, the system needs to have a different analytical system model compared to the conventional imaging system with a lens. We introduce a system model to analyze the resolution and dynamic range (DR), while the model in [43, 62] cannot provide the design trade-off analysis. Note that all the required design parameters for the lensless microfluidic imaging system model are summarized in Table 3-1.

Table 3-1 List of key parameters for contact microfluidic imaging system model.

Symbol	Property
T_h	Throughput
F	Frame rate
R	System resolution
D_{PIX}	Actual sensor pixel size
D_{SR}	Effective SR pixel size
d_{obj}	Object distance
v	Microfluidic flow speed
n	SR magnification factor
M	Number of LR frames
A	Microfluidic channel cross-sectional area
L	Microfluidic channel length

σ	Resolution factor
j	Intensity factor
C	Image contrast
I	Image intensity
a	Contrast amplitude
d	Characteristic distance
b	Shape parameter
ε	Dynamic-range factor
L_1	Row number of the sensor array
L_2	Column number of the sensor array

The overview of the lensless microfluidic imaging system model is shown in Figure 3-5. A microfluidic channel with a cross sectional area A is located in close proximity above a CMOS image sensor array. The object distance to the sensor array is assumed to be d_{obj} . The samples to be imaged are flowing with the fluid at a flow speed of v from the inlet to the outlet in the microfluidic channel. With the light source from the top, the sample images are directly projected to the sensor array. The image information is captured by the CMOS image sensor pixels, which are assumed to be square and of a D_{PIX} length.

Figure 3-5 Contact microfluidic imaging system model.

3.2.1 Resolution

Figure 3-6 Resolution model for the lensless microfluidic imaging system, which shows the captured image intensity profile in one dimension.

For the lensless microfluidic imaging system in Figure 3-5, the image is projected directly by the object onto the CMOS image sensor. Due to the diffraction effect, the center part of the image shows a higher intensity and the intensity for the peripheral part is lower. As shown in Figure 3-6, the intensity profile $I(x)$ of the projected image in one dimension can be assumed as a Gaussian distribution [44, 61]

$$I(x) = \frac{j}{\sqrt{2\pi}\sigma} e^{-\frac{x^2}{2\sigma^2}} \quad (3-1)$$

where σ is the resolution factor, j is the light intensity factor, and x is the pixel coordinate, all to be characterized from the specific setup of the microfluidic imaging system. The Full-Width-at-Half-Maximum (FWHM) under one image intensity profile can be utilized to represent the imaging system resolution R [81] by

$$R = FWHM = 2\sqrt{2\ln 2}\sigma. \quad (3-2)$$

Since the resolution and contrast are relevant, if one defines the image contrast C as

the maximum intensity in the projected image intensity profile, i.e., $C = I(0) = j/\sqrt{2\pi}\sigma$, the relationship between system resolution R and contrast C thus becomes

$$R = 2\sqrt{\frac{\ln 2}{\pi}} \cdot \frac{j}{C}. \quad (3-3)$$

Note that the image contrast C has a relationship with d_{obj} , the distance between the object and the sensor. As shown in [43], one can have

$$C = \frac{j}{\sqrt{2\pi}\sigma} = \frac{a}{1 + \left(\frac{d_{obj}}{d}\right)^b} \quad (3-4)$$

where a , b , d , j are all constants to be characterized from the setup of the lensless microfluidic imaging system. Once the microfluidic channel is placed close to the sensor within the characteristic distance d , the best achievable system resolution R stays small; but when d_{obj} becomes larger than d , R is exponentially degraded, as illustrated in Figure 3-7(a).

Figure 3-7 (a) The relationship between object distance and system resolution. (b) The relationship for sensor dynamic range and pixel size.

As shown in Figure 3-6, the best achievable system resolution R is defined when successfully covering the FWHM with the minimum numbers of pixels. As such, one can determine the distance between two pixels each with effective pixel size D_{PIX} from x_{-1} to x_1 , which can be thought of as using pixels to sample and digitize the image intensity profile. The minimum number of three pixels is usually enough to capture the peak intensity but also the two points at the FWHM. Therefore, the optimal spatial resolution R is obtained as the length of $2D_{PIX}$. Thus, the best system resolution R under a specific d_{obj} is given by

$$2D_{PIX} \leq FWHM = R \quad (3-5)$$

or

$$D_{PIX} \leq \frac{R}{2}. \quad (3-6)$$

Therefore, a smaller pixel size D_{PIX} can result in a much easier case to satisfy (3-6) for design consideration of resolution.

3.2.2 Dynamic Range

Note that [43] assumes that the pixel dynamic range (DR) performance is good enough to cover the image intensity range, and the pixel size D_{PIX} is smaller than FWHM of the intensity profiles, i.e., resolution R . However, even if the object distance d_{obj} is small, it does not guarantee that the best achievable resolution R can be reached in a lensless microfluidic imaging system due to the low-light condition. In fact, one needs to consider both of resolution and DR for a CMOS image sensor design [82-84].

DR quantifies the ability of a CMOS image sensor to response under both bright highlight and dark shadow conditions. It can be defined as the ratio of the largest current signals i_{max} to the smallest detectable current signal (or noise) i_{min} [85],

$$DR = \frac{i_{max}}{i_{min}} = \frac{q_{max} - i_{dc}t_{int}}{\sqrt{\sigma_r^2 + qi_{dc}t_{int}}} \quad (3-7)$$

where $q_{max}=C_{PD}V_S$ is the well-capacity, C_{PD} is the photodiode capacitance and V_S is the voltage swing; q is the electron charge; i_{dc} is the dark current; t_{int} is the integration time; σ_r^2 is the variance of the temporal noise (or KTC) [85]. Note that in (3-7) only the pixel-size dependent noises are considered. Other pixel noise such as the frequency-dependent 1/f noise (flicker noise) is not considered for i_{min} .

Since photodiode capacitance C_{PD} , dark current i_{dc} and KTC noise all increase approximately linearly with pixel size D_{PIX} , i.e., $C_{PD}=C_j \cdot D_{PIX}$, $i_{dc}=I_d \cdot D_{PIX}$ and $\sigma_r^2=KT C_{PD}/q^2$, DR increases roughly as the square root of pixel size $\sqrt{D_{PIX}}$ [85, 86] by

$$DR = \frac{C_j D_{PIX} V_S - I_d D_{PIX} t_{int}}{\sqrt{KTC_j D_{PIX} / q^2 + q I_d D_{PIX} t_{int}}} = \varepsilon \sqrt{D_{PIX}} \quad (3-8)$$

where C_j and I_d are the unit junction capacitance and dark current for the photodiode, and ε is the DR factor. Note that the parameters such as C_j , I_d and ε are all process dependent that can be characterized.

Note that the pixel DR needs to cover the maximum image contrast $C=I(0)$. Otherwise, even when the object distance d_{obj} is very small, high contrast images cannot be captured due to the poor DR. As a result, one can have

$$DR \geq C = \frac{j}{\sqrt{2\pi}\sigma} = \frac{2\sqrt{\ln 2} \cdot j}{\sqrt{\pi} \cdot R}, \quad (3-9)$$

or

$$D_{PIX} > \frac{4\ln 2 \cdot j^2}{\pi \cdot R^2 \cdot \varepsilon^2}. \quad (3-10)$$

Therefore, a larger pixel size D_{PIX} can result in a much easier case to satisfy (3-10) for design consideration of dynamic range.

Chapter 4

CMOS CMUT Sensor for Ultrasound Imaging

4.1 CMUT Sensor and High-frequency Ultrasound Imaging

Among the existing biomedical imaging modalities such as X-Rays [30], computed tomography (CT) [31], and magnetic resonance imaging (MRI) [32], the research and development of ultrasound medical imaging systems has gained much interest in recent years due to its non-invasive diagnosis capability, much less harmful characteristics to the human body, lower cost, and portability [87]. In addition, the emergence of capacitive micromachined ultrasound transducer (CMUT) [88-90] has helped to increase the usage of ultrasound medical systems to the medical community even further. CMUT is basically a transducer that is operated upon electrostatic force and converts ultrasound acoustic waves into electrical signals, and vice versa. Compared with conventional piezoelectric ultrasonic counterpart, which has dominated as the ultrasound transducer choice in the past [91, 92], CMUTs demonstrated ease of fabrication into dense arrays, broader fractional bandwidth characteristic for higher axial resolution, and more importantly, low-cost CMOS compatible fabrication capability to allow the integration with front-end transmitting and receiving application specific integrated circuit (ASIC) electronics for more powerful functions [5]. Till now, different types of CMUT devices have been designed and fabricated using surface micromachining [93, 94] and wafer bonding technologies [95, 96]. Most of the application developments for CMUTs have been focused on the 2D and 3D medical imaging [6, 97], non-destructive testing [98], and high density focused ultrasound therapy [28, 99].

Typical ultrasound systems operate at sub-10 MHz frequencies with one-dimensional CMUT array for two-dimensional (2-D) diagnostic imaging applications. High frequency ultrasonic imaging is considered as the next frontier in ultrasonic

imaging because higher frequencies yield much improved spatial resolution for better biomedical diagnostics. The spatial resolution is defined as the ability of an ultrasound imaging system to distinguish two separate points in space, which can be further categorized into lateral resolution and axial resolution. Lateral resolution is defined as the ability of the system to distinguish two points at the same depth from the transducer surface in perpendicular to the direction of the ultrasound wave. It is determined by the ultrasound wavelength and the depth of imaging as follows,

$$R_L = F^\# \lambda = \frac{\lambda \cdot d}{a}. \quad (4-1)$$

where $F^\#$ is the f-number, a is the aperture size (i.e., spatial dimension) of the transducer, d is the focal depth, and λ is the wavelength [36]. Therefore, lateral resolution is best at shallow depths and worse with deeper imaging. The axial resolution (also known as longitudinal resolution) is the resolution in the direction along the length of the ultrasound wave, which is determined by the wavelength (λ) and numbers of pulses (N) as follows,

$$R_A = \frac{N \cdot \lambda}{2}. \quad (4-2)$$

Thus, to improve the axial resolution, the pulse frequency needs to be increased. And it is the same at any points along the wave, i.e., not affected by depth of imaging. From both (4-1) and (4-2), we can see that the wavelength, and consequently the frequency, directly determines the resolution of the ultrasound imaging system. However, as the attenuation rate (dB/MHz/cm) increases with the frequency of ultrasound wave, arbitrarily increasing the ultrasound wave frequency to obtain finer spatial resolution is not desirable. Therefore, in determining frequency, a necessary consideration is the trade-off between the resolution and the penetration distance of the ultrasound wave. Having set the frequency of an ultrasound wave, the specification for the frontend electronics, such as preamplifier bandwidth and ADC speed, can be

determined. Note that the wavelength of a pulse is determined by the operating frequency of the transducer.

Although at the expense of sacrificing penetration depth, high frequency ultrasound imaging still provides a variety of clinical applications such as visualizing blood vessel wall, anterior segments of the eye and skin, glaucoma and ocular tumors diagnosis, lens hardness detection in the eye. The commercially available ultrasound microscope imaging units operate at 50 MHz and provide lateral and axial spatial resolution of approximately 50 μm and 25 μm , respectively [29].

Therefore, in order to obtain better image resolution, higher sensitivity, and signal-to-noise ratio (SNR) for advanced three-dimensional (3-D) imaging systems, an increase in the number of elements in the transducer array are required in 2-D array configuration with higher frequency (>30MHz) operation [100, 101]. The interfacing of the analog front-end (AFE) IC, consisting of both high-voltage (HV) and moderate voltage circuitry, with the tightly-packed multi-channel 2-D CMUT array is a challenge that must be overcome.

In this chapter, we demonstrate a two-channel AFE IC unit cell for large array IC interfacing with high-frequency 2-D CMUT array in 3-D ultrasound medical imaging applications which is a critical part of the imaging system in deciding the overall performance. The IC consists of two high-voltage (HV) pulsers in the transmit path to drive the transducers and a shared high-frequency low-noise preamplifier with HV protection switches to receive the reflected echo signal. Note that we use a transimpedance amplifier (TIA) as the primary component of preamplifier in the AFE receiver, and it is the main focus of the discussion of circuit design. Careful considerations are done in designing such a front-end array along with issues of using high supply voltage with regular supply voltages in a single IC with a constrained silicon area.

4.2 Ultrasound AFE Architecture

The overall architecture of a typical multi-channel ultrasound imaging system is shown in Figure 4-1. The overall system consists of the multi-channel AFE integrated with CMUT transducer array through flip-chip bonding [97] in order to minimize the connection parasitics. For the transmitter side, the HV pulsers in the AFE IC are

driven by low-voltage trigger signals generated by the digital signal processor (DSP) with controlled delays for beamforming [102]. The high voltage pulses from the pulser output excite the CMUT elements, generating acoustic waves into the acoustic medium. While these acoustic signals propagate through the medium, some of them are reflected back as echo signals, due to the difference in the acoustic impedance levels of tissue layers, which are converted back into electrical signals by the CMUT elements, and processed by the receiver front-end. For the receiver side, the low-noise preamplifiers in the AFE are followed by time-to-gain (TGC) compensation amplifiers [103], which typically consist of variable gain amplifiers (VGA) and a digital-to-analog converter (DAC) to control the gain according to the depth of the received echo signal. The anti-aliasing filters follow the TGC, with analog-to-digital converters (ADC) to digitize the signals and connect to the DSP for signal processing and ultimately construct an image from the pulse-echo signal information.

Figure 4-1 Block diagram of a typical ultrasound imaging system, including the multi-channel AFE IC, CMUT array, and signal processor.

Considering flip-chip bond integration between the AFE and CMUT using post-wafer processing, the overall die floor plan of the 2-D multi-channel AFE IC system is presented in Figure 4-2. The AFE IC consists of identical AFE unit cells in a 2-D array along with digital multiplexer and demultiplexer blocks for column/row address and enable control for transmit and receive modes, pulser trigger controls, bias circuits, and output buffers to drive the following external components in the receive path. The size of the array and the allocated unit cell area for flip-chip bond integration is

decided by the CMUT device characteristics which vary on the targeted specific application. In this work, the overall system is ultimately targeted for 3-D ultrasound bio-microscope application [104] to obtain a high resolution image of a patient's eye to diagnose Glaucoma with high frame rate while minimizing the discomfort given to the patient in the aging society [29].

Figure 4-2 Overall system floor plan for 2-D multi-channel analog front-end IC.

The scope of this work includes the two-channel AFE IC, which is an unit cell for multi-array interface front-end IC development, shown in Figure 4-3, consisting of two HV pulsers in the transmitter path and a single low-noise preamplifier in the receive path shared by the two CMUT elements to ultimately minimize the area per channel on the IC chip. This is because the specified layout area per CMUT element on the IC chip underneath the CMUT wafer for flip-chip bonding is $600\ \mu\text{m} \times 600\ \mu\text{m}$, decided by the CMUT device, and it is difficult to integrate one preamplifier per one channel. Between the pulser and the preamplifier, a HV protection switch using HV double-diffused lateral MOS (DMOS) transistor is placed in order to isolate the preamplifier circuit to avoid possible breakdown in the transmit mode and also to select the

individual CMUT element during the echo receive mode. Among several DC biasing schemes for the CMUT, the HV dc biasing for the CMUTs is done through a large external resistor R_B and a shunt capacitor C_B is added for AC ground for the CMUT [101].

Figure 4-3 Two-channel analog front-end IC unit cell consisting of two HV pulsers, two HV protection switches, and a shared low-noise preamplifier.

4.3 Ultrasound AFE Design Specifications

As the AFE is connected with CMUT, all the design specifications are determined by the CMUT device parameters and the corresponding equivalent circuit model. The designed CMUT element consists of 400 unit capacitor cells connected in parallel. Each cell has the dimension of $28\ \mu\text{m} \times 28\ \mu\text{m}$ while the membrane thickness is $3\ \mu\text{m}$ and the vacuum gap size is $0.12\ \mu\text{m}$. One element of CMUT thus has the dimension of around $600\ \mu\text{m} \times 600\ \mu\text{m}$. The centre frequency in immersion is 35 MHz with a fractional bandwidth of 100% while the target focal depth is 6 mm for bio-microscope application, i.e. 12 mm for the total path back and forth. Note that this centre frequency of 35 MHz is determined by targeting a system spatial resolution of $20\ \mu\text{m}$ at the focal depth of 6 mm based on (4-1) and an acoustic transmission speed of 1500

$$\text{m/s in human tissue, i.e., } f = \frac{v}{\lambda} = \frac{v}{R_L \cdot a / d} = \frac{1500 \text{ m/s}}{20 \mu\text{m} \times (20 \times 600 \mu\text{m}) / 6 \text{ mm}} \approx 35 \text{ MHz}.$$

The capacitance variation of the CMUT element due to the acoustic pressure is 2.12 aF/Pa. The maximum acoustic pressure at the surface is calculated to be 3.31e4 Pa, while the returning minimum echo pressure is calculated to be 1.23e2 Pa from the specified focal depth with the attenuation rate of -1 dB/MHz/cm. The corresponding minimal capacitance variation calculated is 2.62e2 aF. This variation in capacitance generates an AC current signal according to the following equation

$$I_{CMUT} = V_{Bias} \frac{\partial(\Delta C)}{\partial t} = \frac{\partial(\Delta Q)}{\partial t} \quad (4-3)$$

where V_{Bias} is the DC bias voltage applied to one of CMUT electrodes for maximum electrostatic force, ΔC is the change in capacitance, and ΔQ is the proportional change in the amount of charge. The design parameters of our custom designed CMUT is listed in Table 4-1. The design and fabrication details of the CMUT element are beyond the scope of this work and will not be further discussed. We simply show the CMUT array diagram and the photo of the CMUT elements in Figure 4-4. The equivalent electrical model of the CMUT element in immersion utilized in the AFE IC interface simulation is shown in Figure 4-5, mainly consisting of a large parallel capacitor and a resistor.

The gain of the receiver front-end preamplifier is specified so that the output of the preamplifier is to produce a maximum of 1V_{P-P} voltage signal to be inputted to the following TGC block when the maximum acoustic pressure signal is received by the CMUT. Using (4-3) for his case, the maximum fixed gain of the preamplifier is 61dBΩ. The preamplifier bandwidth needs to cover 100% fractional bandwidth of the CMUT, i.e., 52.5MHz, a high frequency in ultrasound imaging application. As the input signal to the preamplifier is a current signal, the input referred current noise is important. Using (4-3), for the case when the minimum acoustic pressure echo signal is received, the required input referred current noise of the preamplifier is calculated to be less than 1.15μArms integrated over 35MHz of bandwidth. The output load of preamplifier is 3.2pF//0.31MΩ input impedance of the TGC on PCB. For the transmitter front-end, the HV pulse has a pulse width of 30 ns with a repetition rate at

around 30-35kHz, while generating a $30V_{P-P}$ unipolar HV signal to drive the CMUT in order to produce a large enough acoustic pressure signal to travel to the specified focal depth. The simplified timing diagram for the two-channel AFE operation in transmit and receive mode is shown in Figure 4-6.

Table 4-1 Key design parameters for the custom designed CMUT device.

<i>Parameter</i>		<i>Values</i>
CMUT array (elements)		16×16
CMUT cells per element		20×20
CMUT cell geometrical profile	Width	$28\mu m$
	Depth	$28\mu m$
	Thickness	$3\mu m$
	Gap size	$0.1\mu m$
CMUT element dimension		$600\mu m \times 600\mu m$
CMUT excitation voltage (V_{P-P})		20V
Bandwidth		17.5-52.5MHz
Capacitance variation		2.12aF/Pa
Capacitance per element (deflated)		44pF

Figure 4-4 (a) Diagram of CMUT array, (b) one CMUT element, (c) one CMUT cell, (d) cross-section view of CMUT cell, (e) top view of CMUT cells.

Figure 4-5 Equivalent electrical model for the CMUT device in immersion.

Figure 4-6 Timing diagram of analog front-end operation.

4.4 Circuit Design and Implementation

4.4.1 Process Selection

To radiate a large acoustic pressure from the transducer surface, the CMUT needs to be biased at a high DC voltage and driven by a high voltage pulse signal. In this work, the GlobalFoundries (GF) HV 0.18 μ m Bipolar-CMOS-DMOS (BCD) process is selected which provides a maximum of 30V_{P-P} drain-to-source voltage for HV asymmetrical and symmetrical DN MOS and DPMOS transistors with 0.74 and 0.79 threshold voltages, respectively. To avoid channel hot carrier effects and eventual avalanche breakdown, the vertical length and doping concentration of the n-well at the drain region of a HV DMOS transistor is extended. Therefore, DMOS transistors occupy layout areas that are orders of magnitude larger than nominal transistors. The main limitation of these devices is the low gate-to-source breakdown voltage of 6V compared to an over 30V breakdown between drain and source, which has to be taken into account in the design stage of HV circuits.

In addition to the HV DMOS transistors, 1.8-V, 5/6-V standard NMOS/PMOS transistors are provided for the design of nominal voltage core blocks and allows for both types of transistors to be fabricated on the same die.

4.4.2 High Voltage Pulser

Figure 4-7 shows the overall block diagram of the HV transmitter front-end [105]. The primary requirement of the HV transmitter is to generate a HV pulse signal without compromising the reliability of the operating transistors in the circuit. The transmitter front-end consists of a 1.8-to-5 V level-shifter, which converts the external DSP/FPGA generated 1.8 V_{P-P} trigger signal to swing between 0 and 5 V at the output. Then the 5-V_{P-P} signal is divided into two separate paths. The upper path contains a second level-shifter to convert the signal to swing between 25 V and 30 V in order to drive the gate of DPMOS transistor of the output driver. The lower path, on the other hand, goes through inverter-based delay buffers to drive the gate of DN MOS transistor of the output driver. The output driver is followed by the corresponding CMUT element where the CMUT is driven with a 30-V_{p-p} HV pulse so that an ultrasound signal with sufficient acoustic pressure is generated for propagation through the acoustic medium.

Figure 4-7 Block diagram of transmitter front-end IC.

Figure 4-8 shows the schematic diagram of 1.8-to-5 V level-shifter [105]. The circuit consists of cross-coupled PMOS transistors and two NMOS transistors driven by two complementary input signals noted as IN and INB. When the input voltages IN and INB are low and high, then M_{N1} and M_{N2} are OFF and ON, respectively. Then M_{N2} pulls down node B and M_{P1} is turned ON, which will pull up node A. This will turn OFF M_{P2} and OUT_5Vp-p will pull down to GND. On the other hand, when the input voltages IN and INB are high and low, then M_{N1} and M_{N2} are ON and OFF, respectively. Then M_{N1} pulls down node A and M_{P2} is turned ON, which will pull up node B resulting in OUT_5Vp-p to be at 5 V.

Figure 4-8 Schematic of 1.8-to-5 V level-shifter.

Figure 4-9 Schematic of 5-to-25V level-shifter and HV output driver.

Figure 4-9 shows the schematic of the second level-shifter and the HV output driver to generate 30 V_{P-P} unipolar pulses to excite the transducer [105]. As the DMOS transistors can only sustain 5-V gate-to-source voltage, a second level-shifter is needed to produce a pulse that swings between 25 V and 30 V to drive the DPMOS transistor of the output driver. In order to prevent junction breakdown of the regular MOS transistors during high voltage operation, DMOS transistors are used in both the level-shifter and output driver so that reliability in the circuit operation is maintained. However, the disadvantage of these DMOS transistors is the added process cost, increased layout size and parasitic capacitance. In addition, the device on-resistance of these transistors is larger than regular CMOS transistors and the sizing has to be sufficiently large in order to drive the following CMUT element to a high voltage at megahertz frequencies. Therefore, for this work, standard CMOS transistors are used wherever possible in order to minimize the overall area of the transmitter front-end.

The output driver stage consists of DMOS transistors M_{HVP1} and M_{HVN1} , in which the gates are driven by two signals created from the regular voltage triggering logic. The gate of M_{HVN1} is controlled by Pulse_trig3, which swings between ground and 5-V. On the other hand, the control signal at node B driving the gate of M_{HVP1} needs a

level shift to operate between V_{DD30} (30 V) and $V_{DD30}-5$ V, which is made possible by the transistors $M_{HVN2,3}$ and M_{P1-5} . 5- V_{P-P} trigger signals go through a digital logic block consisting of delay cells to create non-overlapping trigger signals *Pulse_trig1* and *Pulse_trig2*. *Pulse_trig1* is used to control the unipolar HV pulse applied across the transducer element, while *Pulse_trig2* with phase delay is used to completely turn OFF M_{HVP1} during the pulse repetition time. These two signals are used to drive the gate of M_{HVN2} and M_{HVN3} , which will have the A and B nodes to swing between 25 and 30 V due to the diode connected transistors M_{P1} and M_{P4} connected in parallel to the M_{P2} and M_{P3} transistors. The signal at B node will then switch M_{HVP1} ON and OFF to apply the 30 V to the output which excites the CMUT. As the transmitter core circuits are mainly designed by the collaborators, in the following, we'll focus on the discussion of TIA based preamplifier design.

4.4.3 Preamplifier

Among several choices for the low-noise preamplifier such as resistive feedback type transimpedance amplifier (TIA) [6, 97], and capacitive feedback charge amplifier [106, 107], a resistive feedback TIA was selected for low-noise detection [4], which has ease of biasing and capability to reach high bandwidth [5, 108]. The core schematic for TIA is shown in Figure 4-10. The TIA is composed of a single-ended amplifier and a feedback resistor R_f . The single-ended amplifier consists of a common-source amplifier followed by an N-type source follower. The TIA acts as a current-to-voltage converter, which has a low input impedance, making it well suited for high-impedance sources and hence maximizing the received input current. The input stage of TIA is the CMUT. During the design simulation, the CMUT equivalent circuit in Figure 4-5 was used.

To maximize the input current, the input impedance R_{IN} must be minimized, with $R_{IN} = R_f / (A + 1)$, where A is the open-loop gain of the TIA. The value of the feedback resistor R_f and the size of the main input transistor $MN1$ is critical in deciding the gain, bandwidth, and the noise performance. The close-loop gain of the TIA is set by the feedback resistance R_f of 1.15K Ω , which is translated to a transimpedance gain of 61.18dB Ω .

Moreover, the -3dB bandwidth of TIA is dominated by the capacitance in the large CMUT at the input of TIA by

$$\omega_{TIA,-3dB} = \frac{1}{R_{IN}(C_{CMUT} + C_{parasitic})} \quad (4-4)$$

where $C_{parasitic}$ is the parasitic capacitance in parallel with the feedback resistor. Note that the primary noise sources of the TIA are from the common source amplifier and the feedback transistor. With the dominant pole at the input and additional poles at the drain nodes of the transistors MN1 and MP3 and at the output, the phase margin in the open-loop configuration is simulated to be over 55° with a load of $3.2\text{pF}/0.31\text{M}\Omega$ representing the input impedance of the following TGC. The simulated frequency response of the closed-loop preamplifier is shown in Figure 4-11. The simulated 3-dB bandwidth is around 100 MHz.

Figure 4-10 Resistive feedback TIA schematic.

In addition, the input referred noise current of the TIA can be represented as

$$\overline{i_{N_in_total}^2} = \overline{i_{N_amp}^2} + \overline{i_{R_f}^2} + \overline{v_{N_amp}^2} \times \left(\frac{1}{R_{in_amp}} + \omega C_{in} + \frac{1}{R_f} \right)^2 \quad (4-5)$$

where $\overline{i_{N_amp}^2}$ and $\overline{v_{N_amp}^2}$ are the input referred current and voltage noise of the core amplifier, $\overline{i_{R_f}^2}$ is the noise of the feedback resistor, R_{in_amp} is the input resistance of the core amplifier, and C_{in} is the total input capacitance including the CMUT capacitance and the input parasitic capacitance. Note that $\overline{i_{N_amp}^2}$, $\overline{v_{N_amp}^2}$ and $\overline{i_{R_f}^2}$ are determined by

$$\overline{i_{N_amp}^2} \approx (\omega C_{in})^2 \frac{4kT\gamma}{g_{m1}}, \overline{v_{N_amp}^2} \approx \frac{4kT\gamma}{g_{m1}}, \overline{i_{R_f}^2} = \frac{4kT}{R_f}. \quad (4-6)$$

As such, the transconductance g_{m1} of MN1 and the value of R_f must be maximized to suppress the noise while considering the bandwidth requirement.

Figure 4-11 Simulated closed-loop frequency response.

The primary noise sources of the transimpedance amplifier are the noise of the main transistor MN1, which dominates the noise of the core amplifier, and RF. The transconductance g_{m1} of MN1 and the value of RF must therefore be maximized to suppress the noise while considering the bandwidth requirement. The simulated input referred current noise at typical operating conditions is 17.5pA/sqrt(Hz) at 35MHz shown in Figure 4-12, which corresponds to a 0.09μArms integrated over 35MHz bandwidth. The total power consumption of the preamplifier is 13mA at 6-V supply

voltage. Due to the existence of a large CMUT capacitance at the input of around 44pF, sufficient amount of current has been used to meet the bandwidth and output swing requirement.

Figure 4-12 Simulated input referred current noise.

Due to the area constraint of the CMUT element described previously, one preamplifier is shared by two CMUT elements, and thus two HV DN MOS switches are connected to the input of the preamplifier so that a selection can be achieved between the two while isolating the preamplifier from the HV pulser output during the transmitting mode. The sizing of the switches are done considering the switch-on resistance R_{ON} , as the gain loss and noise performance are affected with small sizing, while a large switch with area-hungry DMOS transistors consume much die area.

4.5 Measurement Results

4.5.1 AFE IC Characterization

The two-channel AFE IC cell is fabricated in one-poly six-metal (1P6M) HV 30-V 0.18- μm BCD process. The chip microphotograph is shown in Fig. 3.11. Moreover, the 2D array of the two-channel analog front-end cells has also been fabricated using the same process as shown in Figure 4-14. In order to support large dynamic current consumption for the high voltage pulser during transmit mode operation, multiple

wide top metal paths stacked from metal-1 to metal-6 layers are used for the 30-V high voltage supply and ground routing lines. In addition, excessive number of custom-made pads located at the top and right side of the chip is used for the 30-V high voltage supply and ground pins. The total chip area of the core is $1.2\text{mm} \times 0.6\text{mm}$ including the two HV pulsers, one preamplifier, two HV protection switches, and all the routing paths for the control/trigger signals and power/ground lines. The two inputs of the shared preamplifier and the two outputs of the pulsers are placed as close as possible to the two input/output bonding test pads (mimicking the $100\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$ flip-chip bonding pads for CMTU element connection) to minimize the parasitic along the interconnection. The die is housed in a QFP32 package for measurement on a FR4 PCB. For the AFE IC-only characterization without the CMUT, an equivalent CMUT model is assembled on the PCB using surface mount type passive components which can sustain high voltage of up to 50V.

Figure 4-13 Chip microphotograph of two-channel analog front-end IC.

Figure 4-14 The fabricated 16×16 2D array of ultrasound analog front-end cells.

First, the HV transmitter is tested using an external arbitrary waveform generator used as the input trigger. Figure 4-15 presents the $1.8V_{P-P}$ input signal versus HV output pulse measurement capture. A pulse width of 33 ns and repetition rate of 500ns for the 1.8V input trigger pulse is used in this measurement example. A delay of 16.2ns is measured between the input trigger pulse and the $30V_{P-P}$ HV output pulse while driving the total load capacitance of 43pF at the pulser output which meets the specifications.

Figure 4-16 shows the measured frequency response of the preamplifier in comparison with the simulation results. In order to measure the transimpedance gain, an off-chip resistor is placed in series on the PCB at the preamplifier input and the voltage across it is measured by using an active probe to calculate the input AC current. Over 62dB Ω of transimpedance gain is obtained while the 3-dB bandwidth is 75MHz, which closely follows the post-layout simulation results. The offline calculated input noise current density from the measured output noise voltage density is plotted in

Figure 4-17. The measured results closely follow the simulation results and satisfy the required specifications. Table 4-2 summarizes the measured transceiver front-end IC performance.

Figure 4-15 Measured transient response of the HV pulser.

Figure 4-16 Measured TIA gain and bandwidth simulation vs. measurement results.

Figure 4-17 TIA input referred noise measurement results.

Table 4-2 Summary of the measured transceiver front-end IC performance.

Parameter	This Work
Blocks	Preamplifier/ HV Pulser
Preamplifier Gain	62dB Ω
Preamplifier input noise current	27.5pA/ $\sqrt{\text{Hz}}$
Preamplifier current consumption	13mA @ 6V
HV Pulser output voltage	30Vpp unipolar
HV Pulser current consumption	300mA dynamic/ 28mA static
HV Pulser input trigger pulse width	28-35ns
Area	0.7mm ²
Technology	30V HV 0.18 μm BCD

4.5.2 Acoustic Transmission Testing with AFE IC and CMUT

Figure 4-18 Acoustic transmission testing setup for implemented AFE IC and fabricated CMUT sample.

Next, the acoustic transmission experiment is carried out with the AFE IC and in-

house developed high-frequency CMUT elements. CMUT samples with center frequency at 10MHz are used for the testing instead of the 35MHz CMUT samples due to fabrication yield problems. The block diagram and photo of the acoustic transmit testing setup is shown in Figure 4-18. The CMUT sample is mounted on a separate PCB and is connected to the transmitter IC through board-to-board wire connection. The CMUT-mounted PCB is placed in a glass container filled with vegetable oil to mimic underwater environment while the IC mounted board is placed outside. A hydrophone is placed at a close distance away from the CMUT in oil to measure the resulting transmitted acoustic pressure and convert it to a voltage signal. The external arbitrary waveform generator is again used to generate 10MHz 1.8V_{P-P} input pulse and is amplified to a 30V_{P-P} pulse signal by the AFE IC in transmission mode to drive the CMUT at the output. The CMUT converts the electrical signal to an acoustic pressure signal corresponding to the amount of applied voltage. A 20V DC bias voltage is applied to the top electrode of the CMUT for the testing. Figure 4-19 shows the measured acoustic pressure-to-converted voltage at the hydrophone output. The results show a successful transmission demonstration of the proposed AFE IC while driving a CMUT in an acoustic environment.

Figure 4-19 Measured hydrophone output voltage signal.

4.5.3 Acoustic Pulse-Echo Testing with AFE IC and CMUT

Figure 4-20 Acoustic pulse-echo testing setup for implemented AFE IC and fabricated CMUT sample.

Lastly, the acoustic pulse-echo experiment is carried out with the developed AFE IC and CMUT sample. The basic idea is to transmit an acoustic pressure signal from the transmitting CMUT in oil and receive the echo signal resulting from the reflection due to the oil-air layer interface using the receiving CMUT. The block diagram and photo of the acoustic transmit testing setup is shown in Figure 4-20. In this setup, two separate AFE ICs are used for the testing. The first AFE IC is used to drive the transmitting CMUT with a HV signal while the second AFE IC is placed closely to the

receiving CMUT in oil to amplify the weak current signal resulting from the echo signal. The pulse-echo measurements are plotted in Figure 4-21, where the first and second echo reflections are measured. The change in the depth of the oil inside the water tank results in a longer distance between the immersed CMUT and the oil-air surface, resulting in an increased delay of the received echo as shown.

Figure 4-21 Measured pulse-echo response.

This confirms a successful demonstration of the proposed high-voltage, high-frequency two-channel analog front-end IC cell for CMUT interface, and can be utilized as a unit cell for future 2D multi-array AFE IC development for 3D non-invasive high-resolution ultrasound imaging system.

4.6 Conclusions

A two-channel analog front-end IC integrating with high-frequency CMUT array is implemented using high-voltage $0.18\mu\text{m}$ Bipolar/CMOS/DMOS process. The electrical functionality of the proposed AFE IC is characterized in which a delay of 16.2ns is measured between the 33ns input trigger pulse and the HV output pulse

while driving the load capacitance of 43pF from 0 to 30 V. The low-noise preamplifier achieves over 60dB Ω transimpedance gain with 27.5pA/sqrt(Hz) input referred noise current at 35-MHz. The acoustic functionality of the proposed AFE IC has also been verified through the pulse-echo measurement in an oil-immersed environment using our in-house developed CMUT device sample. The developed IC can be utilized for various high performance multi-array ultrasound medical imaging systems, and it provides a device level study for the non-invasive ultrasound imaging towards personalized bio-imaging diagnosis.

To clarify the contributions, as this is a collaborated project with Institute of Microelectronics, Agency for Science, Technology and Research (A*STAR) Singapore, I mainly focused on the preamplifier circuit design, tapeout, and testing. The CMUT device and HV pulser are designed by the collaborators.

Chapter 5

CMOS ISFET Sensor for Ion Imaging

In this chapter, we discuss a dual-mode CMOS ISFET sensor for ion imaging, which is targeted for high-throughput and accurate DNA sequencing application [109]. DNA sequencing has profound impact on life technologies such as drug development, personal genome study, and detection of the genes that are potentially associated with some diseases such as the Parkinson. [11, 110]. The traditional optics based DNA sequencing by fluorescence labelled imaging [111] is time-consuming with bulky optics. Among the non-optical DNA sequencing approaches, ion-sensitive field-effect transistor (ISFET) based CMOS chemical sensor has gained more attention recently [68-71, 112, 113]. As discussed in the background section, ISFET based sensors can detect the ion change (pH) by exploiting the silicon nitride passivation layer in unmodified CMOS technology as the sensing membrane. Therefore, if one DNA slice is attached to a microbead at one microwell, the detected local pH value change can be used for the result of the DNA sequencing. As a result, CMOS-based ISFET pH sensor has been developed for portable and high-throughput DNA sequencings.

Figure 5-1 existing large ISFET array for DNA sequencing, which shows the problem of sequencing accuracy due to pH crosstalk and V_T variation.

However, the problem of significant false sequencing data can exist as shown in Figure 5-1. Firstly, local pH response needs to be correlated to the physical location that contains one microbead with attached DNA slice. If there is no microbead in the microwell, due to cross-talk from neighboring microbeads in the solution, there is still false pH value reported. Secondly, pH variation of large-arrayed ISFET sensor can be large due to pixel-to-pixel threshold voltage V_T mismatch, or fixed pattern noise (FPN).

To tackle the first problem, microscope imaging of the 2D microbead distribution can be applied, which still requires bulky optics. For the second problem, UV exposure is used in [114] to remove the trapped charge accumulated on the gates [114] and back-end digital correction is developed in [115], but they are still not applicable for large-arrayed design. A reference-electrode based differential readout method is developed in [116], which makes differential measurement of a reference FET (REFET) device and ISFET to reduce the noise. However, because REFET device and ISFET device are not the same, the noise is not correlated to be removed.

In this chapter, we have developed one 64×64 CMOS ion-image sensor. Firstly, both pH and image sensing are performed with a dual-mode sensor pixel structure which integrates the ISFET with 4T CIS pixel in standard CIS process as shown in Figure 5-2. Since the microbeads are in direct contact with the sensor surface, the imaging of the microbeads can be detected based on the contact imaging principle without lens [117]. As such, an accurate pH-image correlation map can be generated to prune the false pH values. Moreover, correlated-double-sampling (CDS) is developed to support both pH and optical modes in the sensor readout circuit to reduce the pixel-to-pixel V_T mismatch, i.e., FPN. The CMOS ion-image sensor is fabricated through standard $0.18\mu\text{m}$ TSMC CIS process with an area of $2.5\text{mm} \times 5\text{mm}$. Measurements show a sensitivity of 103.8mV/pH , FPN reduction from 4% to 0.3% and readout speed of 1200fps.

5.1 ISFET Sensor and Ion Imaging

5.1.1 ISFET based DNA Sequencing

The threshold voltage V_T of CMOS based ISFET device can be expressed by [112],

$$V_T = V_{ref} - \varphi_{int} + \chi_{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{OX} + Q_{SS} + Q_D}{C_{OX}} + 2\Phi_F \quad (5-1)$$

where V_{ref} is the reference-electrode potential, χ_{sol} is the dipole potential of water molecules in the solution, and φ_{int} is the potential across the electrolyte-insulator interface, which depends on the pH (H^+ ion density) of solution.

During sequencing, ISFET sensor detects H^+ (or pH) released by DNA polymerase synthesis. A long DNA chain is first fragmented into slices and clonally amplified onto reaction carrier, i.e., microbeads, which are distributed into microwell array on top of the ISFET sensor array. Then all four nucleotides (ATCG) are provided sequentially to react with the template base of the DNA chain. When they are complementary, the nucleotide is incorporated and the released proton H^+ generates a pH shift in the solution that is proportional to the number of nucleotides incorporated. As such, the measured pH change for one DNA slice at a microbead indicates the relevant DNA sequence of ATCG [68]. When the pH changes for DNA slices are detected at millions of spatially localized microbeads by the large-arrayed ISFET sensor, high-throughput and label-free DNA sequencing can be thereby realized. However, the main challenge is to improve the sequencing accuracy that can remove false pH reporting as well as FPN noise during readout.

5.2 Dual-mode ISFET Sensor

5.2.1 Dual-mode Sensor Pixel

In this chapter, a dual-mode ion-image sensor is developed with a 64×64 ion-image sensor array in standard CIS process. Figure 5-2 compares the schematics for (a) 4T-CIS pixel and (c) ISFET pixel with the proposed (b) dual-mode pixel. In addition, Figure 5-3 shows the dual-mode pixel contains a 4T-CIS pixel to sense the shadow image of microbead by the contact imaging [115]. Meanwhile, the source follower (SF) can work as ISFET to detect pH value at one microbead. The cross-sectional view of the dual-mode pixel layout is in Figure 5-3(a) and the top view is in Figure 5-3(b).

In the optical mode, PD first collects photons and converts them to proportional electrons, which are transferred to floating diffusion (FD) by turning on ‘TX’ switch of M6. The shadow of microbead is detected through the contact imaging. The

corresponding voltage signal for optical image is amplified by SF (M2) and read out through its source under the control of 'ROW' select signal of M3. Since there are multiple rows of pixels that share the same PIXOUT line, the row-select transistor M3 is used to isolate different pixel outputs, and is enabled only when the row is selected for readout. The cascade current source (M4 and M5) provides biasing current and is shared by the whole column for better current matching.

In the pH mode, the poly-gate of SF (M2) is all the way connected to the top metal and Si_3N_4 passivation layer, acting as ion-sensitive membrane of ISFET. Since the change of ion (H^+) concentration (or pH) can cause the proportional V_T change of the SF as shown in (5-1), the corresponding voltage signal is correlated to the pH value that is read out through the source of SF.

Figure 5-2 Schematic and cross-sectional pixel layout of dual-mode sensor for DNA sequencing with microbead by contact imaging and ion sensing.

When the chip area of a CMOS sensor array is fixed, the only way to improve throughput is to reduce the pixel size and increase the pixel number. But as the sensitivity of the optical imaging is determined by the PD area, and the sensitivity for chemical sensing is determined by the top metal area of SF, the pixel size cannot be too small [118, 119]. In this design, we choose a 64×64 array and empirically choose a pixel size of $10\mu\text{m} \times 10\mu\text{m}$. Although it is fairly large compared with common commercial CMOS image sensor pixels (ranging from $2\mu\text{m}$ to $6\mu\text{m}$), this is to incorporate enough area for the top metal of SF for chemical sensing. There is also a need to optimally partition the pixel area between optical imaging and chemical

sensing. As the optical imaging mode is only used to decide the existences of microbeads in the microwells, its sensitivity requirement is not as critical as for the chemical sensing. As such, the PD area can be reduced to provide more are for chemical sensing. To optimally decide the pixel size and partition, a detailed modeling need to be done, which will be our future work.

Figure 5-3 (a) Cross-section layout of the dual-mode CMOS ISFET pixel. The existence of microbead can be detected by contact imaging, and the pH shift caused by nucleotide incorporation can be measured by ion sensing. (b) The top view of the dual-model pixel.

5.2.2 Dual-Mode Sensor Architecture

The top-level ion-image sensor chip architecture and die micrograph are shown in Figure 5-4 (a) and (b), respectively. The ion-image sensor contains a 64×64 CIS-ISFET pixel array. Each pixel is in dual-mode to correlate the local pH value to the existence of microbeads detected by the contact imaging such that the false pH value reporting can be avoided. When one row of pixels is activated by row selection, 64-

column pixel outputs are read out sequentially controlled by column decoder. The correlated-double-sampling (CDS) is further deployed to reduce the pixel-to-pixel ISFET threshold voltage mismatch through the column sample-and-hold (S/H) and the global switched-capacitor opamp. After amplifying the difference of reference and signal voltages, a 12-bit pipelined A/D converter converts the results to digitally coded outputs that correspond to pH values.

Figure 5-4 (a) Ion-image sensor chip architecture and (b) die micrograph.

5.2.3 Dual-mode Sensor Control

Based on the dual-mode pixel and sensor structure, the dual-mode sensor control is operated as follows corresponding to the readout timings shown in Figure 5-6.

- When reaction carrier microbeads are initially distributed into the sensor pixel array, the readout timing is set to optical mode shown in Figure 5-6(a) as a normal

4T-CIS pixel. The shadow image of microbead can be captured by contact imaging. After that, the existence of microbead at each pixel can be determined with an address generated.

- Then the optical mode is changed to pH mode. Before loading ATCG solution, the reference reset-signal for the whole pixel array is read out using the timing in Figure 5-6(b).
- After loading ATCG solution, the pH readout timing is changed to Figure 5-6(c) to obtain the signal of the pixel array with actual pH value at one microbead.

As such, one can obtain the accurate correlation between the measured pH data and the distribution of microbeads. The false pH data at empty microwells can be thereby eliminated by the dual-mode sensor. Each pixel output voltage after readout is further amplified and digitized through the column S/H, global amplifier and pipelined ADC in the CDS readout chain shown in Figure 5-5.

5.3 CDS Readout with Suppressed FPN

Figure 5-5 CDS readout schematic for dual-mode sensor.

Correlated-double-sampling (CDS) is commonly used in CIS design to reduce the pixel-to-pixel variation and improve the signal-to-noise ratio during readout. As pixel-to-pixel V_T mismatch (or FPN) significantly affects the readout accuracy of large-arrayed sensor, in this brief, we are the first to deploy the CDS for the V_T mismatch

cancellation and FPN reduction in the ISFET pixel array under dual-mode. The CDS readout schematic supporting both pH and optical modes is shown in Figure 5-5, and the corresponding timing is shown in Figure 5-6.

Figure 5-6 CDS readout timing diagram for both (a) optical mode and (b) pH mode.

Note that that the timing diagram in Figure 5-6 is for readout only. The exposure phase with PD reset through TX pulse is not illustrated. Moreover, the developed CDS is applied to suppress pixel-to-pixel V_T mismatch by using each pixel itself as reference, which is intrinsically better than the differential measurement using another REFET device in [116].

5.3.1 CDS for Optical Sensing

The CDS readout circuit for CIS is realized through the signal chain of CIS pixel, column S/H and switched-capacitor amplifier. As the timing shown in Figure 5-6(a), during the pixel to column readout period, 'CLAMP' switch is on so that the top plate of sampling capacitors C_{SS} and C_{SR} are clamped to V_{CM} . The charges on the pixel output line are sampled to C_{SR} when 'SHR' switch is on and to C_{SS} when 'SHS' switch is on. Then during the column to amplifier readout period, the 'CLAMP' switch is off. The crow-bar switch 'CB' is off during the amplifier reset phase and is on during the charge amplifying phase. The sample and hold (S/H) capacitor value is determined by the balance between the KTC noise and speed. A 1pF poly capacitor is chosen for both

C_{SS} and C_{SR} , i.e., $C_{SS}=C_{SR}=C_S=1\text{pF}$. The output of the column sample capacitors are successively controlled by the column select signals 'COL'.

Following the column S/H is the global switched-capacitor amplifier that consists of one non-overlapping clock generator, a fully differential cascode amplifier with switched-capacitor common-mode feedback (CMFB), and several poly capacitors C_{FS} and C_{FR} for programmable gain control. We also have $C_{FR}=C_{FR}=C_F$.

The non-overlapping clock generator generates a pair of non-overlapping clock signals: ' Φ_1 ' and ' Φ_2 ', and ' Φ_{1P} ' whose falling edge is slightly earlier than ' Φ_1 ' to reduce the possible charge injection and clock feed-through. ' Φ_1 ' and ' Φ_2 ' work under 'CB' to amplify and read out signals of each column. During the reset phase Φ_1 , both inputs and outputs of the amplifier as well as the feedback capacitor C_F are reset to V_{CM} , the common mode voltage. During the amplify phase Φ_2 , the bottom plates of the sample and hold capacitors are shorted by turning on 'CB' for the currently selected readout column; and the feedback capacitor C_F are connected to the amplifier output. Thus, charges are essentially moved from the column sample and hold capacitor C_S to the feedback capacitor C_F . As the two input nodes of the differential amplifier connect with the reset level V_{RST} and signal level V_{SIG} , thus only the difference between them is amplified and output, i.e.,

$$V_{OUT} = V_{OUTP} - V_{OUTN} = \alpha \cdot (C_S / C_F) \cdot (V_{RST} - V_{SIG}) \quad (5-2)$$

where α is the gain of the source follower. As such, the V_{OUT} removes the dependence on V_T for CIS in the optical mode.

5.3.2 CDS for Chemical Sensing

We add switches 'ISFR'/'ISFS' as shown in Figure 5-5 to realize the CDS for ISFET in pH mode. Other readout circuits remain the same. The corresponding timing diagram is shown in Figure 5-6(b)-(c). The CDS readout for ISFET is performed as follows.

As shown in Figure 5-5, before loading solution with microbeads, 'RST' is turned on, and the reset voltage V_{RST} is stored at sampling capacitor C_{SR} by turning on 'SHR'. Meanwhile, 'ISFR' is turned on to force $V_{SIG}=V_{CM}$. As such, the amplifier output is

$$V_{OUT1} = V_{OUTP} - V_{OUTN} = \alpha \cdot (C_S / C_F) \cdot (V_{RST} - V_{CM} + V_T) \quad (5-3)$$

Afterwards the reset voltage level for the whole array is read out and digitized by the 12-bit pipelined ADC at the next stage and saved by the external storage. The corresponding timing diagram is shown in Figure 5-6(b).

After loading solution with microbeads, 'ISFS' is turned on to force $V_{RST}=V_{CM}$. The amplifier output now is

$$V_{OUT2} = V_{OUTP} - V_{OUTN} = \alpha \cdot (C_S / C_F) \cdot (V_{REF} - V_{CM} + V_T - dV), \quad (5-4)$$

where dV is the threshold voltage change caused by the chemical reaction between the ion and the passivation layer; and V_{REF} is the voltage of the reference electrode. This output is also converted by the ADC and readout to the external storage for further digital processing.

As a result, we subtract the two outputs and obtain the difference by

$$V_{OUT1} - V_{OUT2} = \alpha \cdot (C_S / C_F) \cdot (V_{RST} - V_{REF} + dV), \quad (5-5)$$

which removes the dependence on V_T for ISFET in the chemical mode.

5.3.3 Global Amplifier

As for the global amplifier, the feedback capacitors C_F are adjustable among 1pF, 0.5pF and 0.25pF such that the gain C_S/C_F can be selected among 1X, 2X, and 4X under different input signal levels. As such, the sensitivity or dynamic range can be improved. The amplifier utilizes a telescopic structure as shown in Figure 5-7. AC simulation results in Figure 5-8 show an open-loop gain of 68dB and bandwidth of 628MHz. The high GBW enables high-speed readout with 10MHz column-wise readout speed.

Figure 5-7 The global core amplifier circuit.

Figure 5-8 The global amplifier AC simulation results.

5.3.4 Pipelined ADC

The sensed signal by ISFET-sensor array is digitized by 12-bit pipelined ADC before final output. The ADC consists of sample-and-hold input stages, ten serially connected 1.5-bit pipeline stages, and one 2-bit flash stage as shown in Figure 5-9. The digital correction block creates a 12-bit output code by redundant signed digit (RSD). The 1.5-bit per-stage is chosen because of its immunity to the offsets. A telescopic operational amplifier with gain-boosting is chosen for high dc gain, high GBW and fast settling time. The simulation results of DNL and INL of the pipelined ADC is shown in Figure 5-10, respectively. The maximum DNL is 0.44LSB and the maximum INL is 0.61LSB. The ENOB is 11.4 bits, and the SNDR is 70.35dB. As such, the whole row-readout time is $13\mu\text{s}$, including the pixel sampling, amplification and digitization. Therefore, for a 64×64 array, the whole readout time for 64 rows is $64\times 13\mu\text{s} = 0.832\text{ms}$ with a frame rate about $1/0.832\text{ms}=1200\text{fps}$. Fast readout speed can enable us to capture a chemical image in a shorter time. For DNA sequencing, sampling the signal at high frequency relative to the time of the nucleotide incorporation signal allows signal averaging to improve the SNR.

Figure 5-9 12-bit Pipelined ADC architecture.

Figure 5-10 12-bit Pipelined ADC INL/DNL simulation results.

5.4 Measurement Results

The proposed dual-mode sensor is fabricated in TSMC 0.18 μm CIS process. The sensor is packaged by liquid-friendly encapsulation with sensing area open. The chip micrograph with architecture and testing system is shown in Figure 5-11. A 3D-printed plastic holder that fits the sensor package is used to fix the AgCl electrode. The design specifications are summarized in the table of Table 5-1.

Table 5-1 Dual-mode sensor chip design specifications.

Parameters	Specifications
Process	Standard TSMC 0.18 μm CIS
Pixel Type	Dual-mode (Optical and Chemical)
Pixel Size	10 μm ×10 μm
Pixel Optical Sensing Area	20.1 μm^2
Pixel Chemical Sensing Area	22.3 μm^2
Array Size	64×64
Die Area	2.5mm×5mm

ADC ENOB	11.4 bits
ADC SNDR	70.35dB
FPN	0.3%
Frame Rate	1200fps
Total Power Consumption	32mA @ 3.3V

Figure 5-11 Micrograph photo of the dual-mode sensor chip and testing setup.

Firstly, the correlated contact image and pH map of microbeads are shown in Figure 5-12. The contact image determines the existence of microbeads and provides

their addressed distribution. The pH map is thereby locally associated with microbeads by pruning out those uncorrelated pH data.

Figure 5-12 The correlated maps of distributed microbeads: (a) contact images; and (b) pH values.

Next, the pH measurement results are shown in Figure 5-13. The pH of solution is changed by adding HCL and NaOH. The readout pH sensitivity of ISFET by CIS process is measured as 26.2mV/pH with amplifier gain=1 and as 103.8mV/pH with amplifier gain=4 as shown in Figure 5-13. The device sensitivity at gain=1 is somewhat lower than the commonly observed response of 45–56 mV/pH for Si_3N_4 , this can be due to the low-pressure chemical vapor deposition (LPCVD) technique for Si_3N_4 at low temperature, which generally cause low-density and porous passivation layer. It can be optimized by the LPCVD at a high temperature or do additional depositions, which are still standard CMOS process [68].

Figure 5-13 Measurement results: (a) pH sensitivity of dual-mode ISFET sensor.

The sensor chip is also calibrated by testing the pH change of a bacteria (*E. Coli*) culture solution at different time intervals. The measurement results by the dual-mode sensor can correlate well with one commercial pH meter (Checker, Hanna Instruments, RI, US) in Figure 5-14.

Figure 5-14 The comparison with commercial pH meter for bacteria (*E. Coli*) culture solution with glucose at different time intervals.

The comparison of readout voltage variations with and without CDS is shown in Figure 5-15. After performing spatial FFT to the readout voltages with respect to the addresses of the sensor array, the mean and peak variations are reduced by 0.17mV and 0.25mV, respectively. The FPN is accordingly reduced from 4% down to 0.3%.

Lastly, the comparison with the state-of-art ISFET sensors is summarized in Table 5-2. The proposed dual-mode sensor shows the state-of-art results: 10 μ m pixel pitch, 64 $\times$ 64 pixel array, fast frame rate of 1200fps, and sensitivity of 103.8mV/pH in standard CIS process. The pixel pitch can be further reduced and the array size can be scaled to 1 million for higher throughput detection, which is our current on-going work.

Figure 5-15 Measurement results: spatial FFT of readout voltage variations (a) with CDS and (b) without CDS readout.

Table 5-2 Comparison of state-of-the-art ISFET sensors.

	[114]	[120]	[121]	[122]	This Work
Process	5 μ m Non- CMOS	0.35 μ m Modified CMOS	0.35 μ m Standard CMOS	0.18 μ m Standard CMOS	0.18μm Standard CMOS
Pixel Size	200 μ m $\times$	12.8 μ m $\times$	10.2 μ m $\times$	20 μ m $\times$	10μm$\times$

	200 μm	12.8 μm	10.2 μm	2 μm	10μm
Array Size	10 $\times$ 10	16 $\times$ 16	64 $\times$ 64	8 $\times$ 8	64$\times$64
Frame Rate	30fps	-	100fps	-	1200fps
Sensitivity	229mV/pH	46mV/pH	20mV/pH	37mV/pH	26.2mV/pH (gain=1) 103.8mV/pH (gain=4)
Dual-Mode	No	No	No	No	Yes

5.5 Conclusions

In this chapter, a dual-mode CMOS ion-image sensor for accurate DNA sequencing is proposed and demonstrated with the state-of-art results. With the dual-mode pixel design to avoid false pH-value reporting by determine microbead existence with contact imaging; and the CDS readout to suppress FPN for large-arrayed sensor, the DNA sequencing accuracy can be significantly improved. Such a dual-mode CMOS sensor design is potential to pave the way for the future LOC personalized diagnosis systems with multimodal bio-imaging capability.

To clarify the contribution, in this work, I mainly worked on the design of readout and control circuits such as the column/row decoder, column S/H and global amplifier for CDS readout, and top-level integration with pad frame. For the testing, I built up the FPGA-based testing system with MATLAB-GUI based timing control, performed the dual-mode testing using microbead solution and *E. Coli* solution, analyzed the data and wrapped up the results with paper writing.

Chapter 6

CMOS Optical Sensor for Microfluidic Contact Imaging

This chapter discusses the optical sensor for microfluidic contact imaging in the application of a microfluidic cytometer. Flow cytometer has been widely deployed in biological research and clinical diagnostics to automatically determine the count or concentration for one or multiple types of cells [123-126]. For example, in HIV monitoring and treatment, counting of CD4+ and CD8+ T-lymphocytes are required for antiretroviral therapy [127]. In immunophenotyping, human peripheral blood samples are analyzed by calculating cell concentrations for platelets, lymphocytes, and monocytes [128]. All these applications demand high accuracy and throughput with the use of flow cytometer. A conventional flow cytometer measurement is performed by passing a narrow stream of cells through a focused laser beam at a rate of thousands of cells per second. The optical signals such as forward scattering (FSC), side scattering (SSC), fluorescent light emission (FL) are simultaneously measured to obtain information such as relative size, granularity or internal composition of cells. However, because of the bulky equipment size with sophisticated optical measurement procedure, the conventional flow cytometer is prohibitive for point-of-care application [129, 130]. In addition, flow cytometry is traditionally relied on non-imaging technique by laser scattering and fluorescence emission for cell identification [131-133] and hence is lack of image information of cells [134, 135].

The recent advance of microfluidics-based LOC technology has introduced the possibility for the miniaturized microflow cytometer for portable flow cytometry [131-133, 136, 137]. With the integration of CMOS image sensor chip underneath the microfluidic channel, microfluidics-based lensless imaging systems [44, 55, 57, 61, 63, 64, 73, 138] can be developed for portable contact-imaging [43] based microflow cytometer. Illuminated by incoherent light source, the direct projected shadow or contact images of cells can be captured by the image sensor underneath without lenses [44, 55, 57, 61, 63, 64, 138].

However, due to this direct-projection based imaging scheme without lens, the spatial resolution of such contact imaging system is directly determined by the pixel size of CMOS image sensor. As such, the captured images of microfluidic flowing cells are intrinsically in low-resolution (LR) with loss of details in cell morphology information as most of the current pixel size of commercial CMOS image sensor ranges from 2 μm to 6 μm . It is intuitively to think of further decrease the pixel size to below 2 μm or even 1 μm . However, scaling the pixel size reduces the amount of light incident on each pixel and puts an upper bound on the photodiode signal [139-141]. Even for a perfect pixel that converts all the collected incident photons into electrons, there is still a limit to pixel size based on the fundamental photon shot noise [141]. Moreover, as the pixel size approaches the wavelength of visible light, the diffraction problem causes a sharp increase in the amount of light that reaches adjacent photodiodes, known as spatial optical crosstalk [142], which degrades the signal at neighboring pixels as well as the resolution. Therefore, another system-level solution is necessary.

Figure 6-1 Different contact imaging systems without optical lens. (A) Static contact imaging system. (B) Microfluidic contact imaging system with capillary flow. (C) The proposed microfluidic contact-imaging cytometer system with continuous flow: (C1) bonding process; (C2) overall system structure.

As shown in Figure 6-1(A), one LUCAS system is demonstrated for cell counting application [55]. To distinguish different cell types, the cell intensity distribution

pattern of raw LR shadow or holographic shadow images are used [55, 57]. The cells to be imaged are statically placed in between cover slides above the image sensor array. Thus, without continuously flowing microfluidic, the total solution volume is limited in each test. In [44, 63], a multi-frame sub-pixel resolving super-resolution (SR) processing is proposed with a high-resolution (HR) cell image recovered by capturing a large number (40 to 100) of subpixel-shifted LR cell images. As shown in Figure 6-1(B), in order to capture subpixel motions in multiple frames, a drop-and-flow is employed to maintain the low flowing speed, usually driven by capillary or electroosmotic flow for precise movement control. Moreover, the storage of multiple cell images to recover one SR image consumes huge hardware resource. Both problems limit the throughput when counting multiple continuously flowing cells.

In this chapter, a contact-imaging based microfluidic cytometer is introduced with extreme-learning-machine based single-frame SR processing (ELM-SR) that can perform recognition and counting of cells in continuously flowing solution [143]. The Extreme Learning Machine (ELM) is a general suite of machine-learning techniques. By assuming randomly generated weights with single hidden layer, the ELM can provide fast solution for the generalized feed-forward neural networks with many applications such as bioinformatics, image processing, feature selection, human action recognition, etc [144]. To our best knowledge, this work represents the first study applying the ELM analysis to achieve single-frame super-resolution for cell imaging. Compared to the single-frame SR by interpolation and sharpening [72] the pattern-recognition based SR [145, 146] can recover high-frequency (HF) components containing details for fine structures in cells. In addition, with randomly generated weights between input layer and hidden layer, the pattern-recognition based SR in this work is based on extreme learning machine (ELM) that can have much less expensive iterative training process for on-line SR image recovering [144, 147]. Here is the flow of the developed single-frame ELM-SR for the contact-imaging based microfluidic cytometer. Static HR cell images obtained from microscope are first classified and stored as off-line HR cell image library, which are utilized to train an ELM-SR reference model. Note that HR cell images in library contain the detailed internal cell structure information with HF components. Then, the on-line single-frame SR processing is performed by employing the ELM-SR reference model to recover the necessary HF components from one on-line LR cell image. The recognition and

counting for different types of flowing cells can be thereby performed accurately by only checking for the strongest structure similarity [148] with reference to the off-line static HR images. As such, the developed microfluidic imaging cytometer can achieve single-cell image quality without flow rate limitation when compared with [44, 63].

We examined the performance of the prototype of the microfluidic cytometer by measuring the absolute number of microbeads in solution per unit time of flow, and the concentration ratio of mixed flowing HepG2 and red-blood cells (RBCs) both at a flow-rate of 5 μ L/min. Less than 8% error is observed for the absolute number of microbeads; and a coefficient variation of 0.10 is observed for the cell ratio when compared with a commercial flow cytometer. The demonstrated microfluidic imaging cytometer is thereby meaningful for rapid counting of various cells for point-of-care diagnosis as well as for water quality analysis in remote and resource-limited areas.

6.1 CMOS Image Sensor and Contact Imaging

6.1.1 CMOS Image Sensor

Figure 6-2 System architecture of the super-resolution CMOS image sensor.

Before the microfluidic contact imaging system integration with microfluidics, a high speed and high DR CMOS image sensor with SR processing is designed. Figure 6-2 shows the overview of the proposed SR CMOS image sensor architecture. The sensor consists of a 128(H)×128(V) 3T active pixel array. A large sized pixel ($10\mu\text{m}\times 10\mu\text{m}$) with 56% fill-factor for high DR is employed. The row decoder and row driver drive out the pixel signals in row by row mode and decide the rolling shutter exposure sequence of the whole pixel array. The column-parallel readout architecture is employed here to achieve high-speed operation. In the pixel readout path, each column has a column amplifier, 10-bit Single-Slope ADC (SS-ADC) and SRAM memory. All the digital timing control in this chip is generated from the on-chip digital timing control block. Before the pixel signals are readout, they are further processed by the on-chip single-frame super-resolution processing block to improve the resolution by 4X and hence compensate the sacrificed spatial resolution. The design details are introduced as follows.

6.1.2 High Dynamic Range Pixel

For biomedical contact imaging applications, high DR is the important factor to be considered. A large pixel size can help reach a high DR. In this work, a $10\mu\text{m}\times 10\mu\text{m}$ n^+/p -sub type pixel is implemented with a fill-factor of 56% with pixel layout shown in Figure 6-3.

Figure 6-3 3T large-sized pixel structure: (a) layout. (b) A-A' cross section.

The pixel is working in the following way. Before the pixel exposure, an initial reset is applied to dump out the charge in the pixel photodiode. When the pixel starts the exposure and integrates light, photons are converted to charge and the voltage across the photodiode decreases. After the integration is finished, the signal output voltage is sampled as V_{SIG} . Then, the pixel reset switch is on, and the reset output voltage is also sampled as V_{RST} . The final pixel output is the voltage difference ΔV between V_{RST} and V_{SIG} .

6.1.3 Column Parallel Readout

As shown in Figure 6-4(a), a column-parallel readout is implemented in this work with correlated-double-sampling (CDS) [41-42]. The corresponding timing diagram of CDS is shown in Figure 6-4(b). For each row of pixels to readout, the ϕ_{SEL} is set to high. During the high level phase of each ϕ_{SEL} , when the ϕ_{RST} is low, the ϕ_{UD} is set to high to have up-counting for the pixel signal level conversion (V_{SIG}); when the ϕ_{RST} is high, ϕ_{UD} is set to low to have down-counting for the pixel reset level conversion (V_{RST}). During the up/down transition, the counter data will be maintained unchanged by the ϕ_{KEEP} control signal. Thus, the pixel signal level and reset level will be automatically subtracted and the real digital pixel information is obtained ($\Delta V = V_{SIG} - V_{RST}$).

There are following three main blocks in the column-parallel readout according to the signal flow.

1) Column Amplifier: The pixel output is first amplified by a gain-adjustable (1X, 2X, and 4X) switch-capacitor column amplifier. To achieve high DR, it can amplify the signal and hence increase the pixel sensitivity; meanwhile, it can also reduce the readout noise as the amplifying stage is close to the pixel photodiode.

2) Single-Slope (SS) ADC: The SS-ADCs have been widely used for high-speed column-wise readout. The 10-bit SS-ADC consists of a ramp generator, a comparator, 10-bit ripple counter and 10-bit data latch in each column. Note that in the current CMOS image sensor implementation, the ramp signal is generated from external for testing flexibility.

In addition, a 10-bit asynchronous counter is employed to perform the A/D conversion by counting the number of clocks until the output of the comparator changes. The counter outputs are the digital codes to represent the pixel signals. The

counting up or down direction is controlled by the ϕ_{UD} such that the digital CDS can be performed within the counter.

3) SRAM data output: Afterwards, the 10-bit counter output data from SS ADC are locked by a 10-bit latch; then are written into a SRAM to drive the final data for off-chip processing.

Figure 6-4 (a) Schematic diagram of one column signal path for column-parallel readout. (b) Digital timing control diagram for the signal readout chain.

6.1.4 Super-resolution Processing

Super-resolution (SR) is one effective image processing technique to reconstruct high-resolution (HR) images [149-152] from observed low-resolution (LR) images.

Assume that the desired HR image is of size $nL_1 \times nL_2$, where L_1 and L_2 are the row and column numbers of the sensor; and n is the down sampling factor or magnification factor, in both horizontal and vertical directions. Thus, the HR image vector when denoted in lexicographic notation is $\mathbf{x} = [x_1, x_2, \dots, x_{nL_1 \times nL_2}]^T$. Similarly for LR images, they can be denoted as $\mathbf{y}_k = [y_{k,1}, y_{k,2}, \dots, y_{k,L_1 \times L_2}]^T$, where $k=1, 2, \dots, M$, and M is the total number of LR frames. The observed LR images usually result from wrapping, blurring, and down sampling operation by [150]

$$\mathbf{y}_k = \mathbf{D}\mathbf{B}_k\mathbf{W}_k\mathbf{x} + \mathbf{V}_k, 1 \leq k \leq M \quad (6-1)$$

where $\mathbf{W}_k$ is a warp-matrix of size $nL_1nL_2 \times nL_1nL_2$, which is obtained through motion estimation between reference images $\mathbf{y}_k$ ($2 \leq k \leq M$) and the chosen current frame $\mathbf{y}_1$ (assuming the 1st LR frame is chosen as the current frame). $\mathbf{B}_k$ is the $nL_1nL_2 \times nL_1nL_2$ blur-matrix, which represents the point spread function of the image sensor. $\mathbf{D}$ is the $(L_1L_2)^2 \times nL_1nL_2$ down sampling matrix, standing for the decimation operation to reduce the number of observed pixels in the measured images. $\mathbf{V}_k$ represents the noise vector.

The aim of SR is to estimate $\mathbf{x}$ based on the known images $\mathbf{y}_k$. In previous microfluidic contact imaging system, a series of images are captured when samples are flowing in a relatively controlled manner due to the laminar flow effect, making it similar to the case of video sequences where the photographed scene is static and the images are obtained with slight translations [44, 63]. Thus, the SR processing for lensless microfluidic imaging becomes a special case, i.e., the warping between the measured images is pure translation so that $\mathbf{W}_k$ is block-circulant; the blurring is space invariant for all the measured LR images, i.e., $\forall k, \mathbf{B}_k = \mathbf{B}$; and the additive noise is white noise, i.e., autocorrelation of the Gaussian random vector $E\{\mathbf{V}_k\mathbf{V}_k^T\} = \sigma_v^2 \mathbf{I}$ [152]. Thus, the Maximum-Likelihood estimation of $\mathbf{x}$ to generate the best HR image can be done as follows [149]

$$\mathbf{x} = \arg \min_{\mathbf{x}} \left\{ \sum_{k=1}^M [\mathbf{y}_k - \mathbf{DBW}_k \mathbf{x}]^T [\mathbf{y}_k - \mathbf{DBW}_k \mathbf{x}] \right\}. \quad (6-2)$$

The working steps of the multi-frame SR implemented for microfluidic contact imaging is illustrated in Figure 6-5. Assume that there are M captured images, which are LR based on the large pixel size D_{PIX} . Each LR image is composed of $L_1 \times L_2$ pixels, where L_1 and L_2 are the row and column numbers. The reconstruction processing includes interpolation, motion estimation, data mapping and deblurring. In M frames of images, we choose one image to be the current frame, and the other $(M-1)$ images to be the reference frames. In order to map all the $(M-1)$ reference LR frames into the current frame and create the final HR frame, the SR processing first needs to interpolate the current LR frame to obtain enough HR pixel grids. Thus the interpolation time defines the final magnification factor n , which is chosen by the user [150]. Since there are $(M-1)$ reference frames, the interpolation time, i.e., magnification factor n , can also be chosen as $(M-1)$. As such, the HR image array size becomes $(M-1)L_1 \times (M-1)L_2$. In the example shown in Figure 6-5, $L_1=L_2=2$, and $n=4$, $M=5$. After mapping the reference frames, the data for the remaining HR grids where no LR frames are mapped will be obtained through bilinear interpolation. Note that more reference frames could be used for the SR reconstruction, but that may also introduce a larger implementation cost.

Figure 6-5 Working principle of multi-frame super-resolution processing.

By merging the LR pixel information from multiple images, multi-frame SR shows a good performance in resolution improvement. However, it requires large memory and hence large latency to store and process both current and reference frames, which becomes more severe when the pixel array is bigger or a larger magnification factor is chosen for SR. In this work, we propose a single-frame SR approach that only utilizes one single LR-frame to reconstruct the HR image with bilinear interpolation processing [72]. The advantage of more efficient memory usage makes this approach possible for on-chip implementation with significantly reduced hardware implementation resource as well as reduced latency. Figure 6-6 shows an overview of the proposed system working principle for the single-frame SR. The digital output data from whole sensor array in terms of L_1 -row and L_2 -column is transferred in serial into a data buffer within the SR block. They are then processed as follows:

Figure 6-6 Working principle of the proposed single-frame SR algorithm.

Step 1: Data pumping. The output data from the first row ($N_1, N_2 \dots N_{L2}$) and the first two pixels of the second row ($(N+1)_1, (N+1)_2$) are pumped into the data buffer array.

Step 2: Data processing. In this step, the first original pixel (N_1) will generate HR pixel data of 4×4 pixels ($N_1, P_1, P_2 \dots P_{15}$) by bilinear interpolation with the other three reference pixels ($N_2, (N+1)_1, (N+1)_2$); then the 4×4 processed pixel data are driven to the SR pixel frame buffer as outputs.

Step 3: Data pumping. The data is continuously pumped in and the second original N_2 moves to the processing position. With another 3 reference pixels ($N_3, (N+1)_2, (N+1)_3$), it will generate another group of 4×4 pixels in a similar fashion and are driven to the SR frame buffer after processing.

When one full LR frame is processed, the corresponding SR image is obtained. As a result, the original data of 128×128 pixel-array with $10 \mu\text{m}$ resolution will generate processed data of 512×512 with $2.5 \mu\text{m}$ resolution. Compared to the multi-frame SR, the single-frame SR requires much less storage with less computation. The whole processing block only needs one processing core and one row data buffer to store the data for processing with significantly reduced latency.

However, due to the small area of the pixel array, the integration with microfluidic channel is difficult for actual flowing cell testing. Thus, we utilize a commercial CMOS image sensor (Aptina MT9M032, San Jose, CA) to build the lensless microfluidic cytometer as discussed below.

6.2 Lensless Microfluidic Cytometer

6.2.1 System Design

The proposed contact-imaging based microfluidic cytometer for flowing cell recognition and counting is shown in Figure 6-1(C). It includes one PDMS microfluidic channel attached on top of a CMOS image sensor, through which cells flow continuously. A syringe pump continuously drives the sample solution of interest into the channel and controls the flow rate. A conventional white LED lamp is applied as the light source above to project flowing microbeads or cells in the solution. The CMOS image sensor can continuously capture shadow images underneath. The captured digital image frames are then rapidly processed with machine-learning based

single-frame SR algorithm, which can improve resolution of shadow images such that one can recognize and count the flowing cells.

To build the contact-imaging based microfluidic cytometer with higher spatial resolution, a greyscale CMOS image sensor (Aptina MT9M032, San Jose, CA) is selected with a pixel size of $2.2\mu\text{m} \times 2.2\mu\text{m}$. The active sensing area is $3.24\text{mm(H)} \times 2.41\text{mm(V)}$ by a $1472\text{(H)} \times 1096\text{(V)}$ pixel array. The hardware design is shown in Figure 6-7(C) and Figure 6-7(D).

Figure 6-7 Microfluidic contact-imaging cytometer system for flowing cell detection, recognition and counting. (A) Cell shadow image by contact imaging. (B) Captured video of flowing cells. (C) CMOS image sensor board schematic with external controls. (D) System board of the developed microfluidic cytometer.

As shown in Figure 6-7(A), the developed microfluidic cytometer is based on contact imaging [44, 55, 57, 61, 63, 64, 138], where the light intensity and contrast of one cell's shadow image is determined by the distance D_{obj} from the object to the pixel array. Note that shorter object distance D_{obj} provides better contrast C_{on} and resolution due to less diffraction effect [43],

$$C_{on} = f(D_{obj}) = A / \left(1 + (D_{obj} / D)^B \right) \quad (6-3)$$

where A is the contrast amplitude, D is the characteristic distance, and B is the shape parameter. Guided by (6-3), we first discuss the design of microfluidic channel and then CMOS image sensor.

Figure 6-8 Microfluidic channel bonding process.

Firstly, the protection glass of the image sensor chip was first removed before bonding with PDMS microfluidic channel, as shown in Figure 6-8. In addition, the microlens layer above the pixel array is removed by treating the sensor under oxygen plasma (PDC-32G, Harrick Plasma, Ithaca, NY) for 45 min (18W) [44]. However, as the developed system utilizes the continuous microfluidic flow, which generates higher pressure to the channel wall than the one using capillary or electroosmotic flow [44, 61], a thin PDMS layer was also spin coated on top of the sensor die. A tight PDMS-PDMS bonding [136] is required as the process shown in Figure 6-1(C1). The spin speed of 9000rpm is set to generate a thickness of $6\mu\text{m}$ [153] for PDMS, as shown in Figure 6-9. Therefore, the object distance of our system is $6\mu\text{m}$ to enable enough contrast for the microfluidic contact imaging. After spin coating and baking, the surfaces of the microfluidic channel and the image sensor were cleaned with ethanol and oxygen plasma and are further bonded together finally as shown in Figure 6-1(C2). Note that after bonding the PDMS coated sensor chip and the microfluidic chip, we also filled the PDMS and curing agent mixture into the gap to encapsulate the bonding wires. As the microfluidic chip fabrication fundamentals are already introduced previously, here we show a schematic diagram of the bonding process in Figure 6-8 without detailed discussion for PDMS channel fabrication.

Figure 6-9 The relation between the PDMS thickness and spin-coating speed and time [153].

Moreover, to make the full use of the active pixel area, the channel length was designed as 4.6mm and cut in diagonal. Thus when bonded on top of the sensor die, the rectangle microfluidic chip was just within the die area of the bonding wire. A relative wide channel width of 500 μ m was chosen such that high concentration of cells can flow through the channel without clogging [154]. The height of the microfluidic channel was 30 μ m, just higher than the normal cell diameters. This ensures that the cells flow close to the sensor surface with better projected image contrast [44]. Besides, in order to improve the wettability of the channel, the channel was coated with bovine serum albumin (BSA) by flowing a 1% solution of BSA in PBS through the channel for an hour [155].

Next, the CMOS image sensor chip was soldered on one low-cost 5.6cm $\times$ 5.6cm printed circuit board (PCB) that provides the sensor with power supplies and digital control signals as shown in Figure 6-7(D). The data transferred from the CMOS image sensor to PC was through a USB interface (CY7C68013-56 EZ-USB FX2, San Jose, CA), which ensures high-speed imaging with maximum data transfer rates of 56 Mbytes per second. The sensor working status such as exposure time, ROI and number of frames to capture was controlled by the status registers that can be accessed through a two-wire serial interface, i.e., SCLK and SDATA, as the schematic shown in Figure 6-7(C). They are set through the custom designed GUI shown in Figure 6-7(B). We set 640 $\times$ 480 image ROI of the sensor to capture the flowing specimens at a sensor frame rate of $\sim$ 200 frames/s (fps).

In the experiments, the microfluidic chip was connected to a syringe pump (KDS Legato180, Holliston, MA) through silastic laboratory tubings and samples were pumped into the microfluidic chamber continuously at a typical flow rate of $\sim$ 5 μ L/min under the illumination from a white light source (Olympus LG-PS2, Tokyo, Japan). The thin tubing of 0.64mm i.d. and 1.19mm o.d. (product no. TW-96115-04, Cole-Parmer, Vernon Hills, IL) was used as it helps reduce dead volume and cell lost compared with thick channel. The light source was placed 12cm above the sensor and the light intensity at the sensor surface was 1.5k Lux. The exposure time of the sensor was set $\sim$ 75 μ s, corresponding to 3 rows of sensor readout time. The readout LR frames were buffered with digital image processing conducted to improve the resolution by single-frame ELM-SR processing. As such, the developed system can automatically recognize and count the flowing cells.

6.2.2 Cell Sample Preparation

In the experiment, HepG2 cells (American Type Culture Collection, MD) were cultured in Minimum Essential Media (MEM) (Gibco, cat# 11095-080) supplemented with 10% fetal bovine serum (FBS) (Gibco, cat# 10270-106), 1 mM sodium pyruvate (Gibco, cat# 11360-070), 0.1 mM MEM non-essential amino acids (Gibco, cat# 11140-050), and grown at 37°C under a 5% CO₂ atmosphere in a T75 flask. The harvested cells were washed and re-suspended in phosphate-buffered saline (PBS). (Fisher Scientific, Pittsburgh, PA). The RBCs were obtained from National University Hospital (NUH) Singapore, also suspended in PBS. The polystyrene microbeads of 6µm diameter (Product# 07312, Polysciences, Warrington, PA) was selected for calibration experiments as it is of similar size with RBC. The microbeads were suspended in PBS. All the samples were sonicated in an ultrasonic benchtop cleaner (Branson 2510E-DTH, Danbury, CT) for 10 minutes before pumping into the microfluidic channel to prevent aggregation.

6.3 Contact Image Processing

Digital image processing is performed to recognize and count the cells flowing through the microfluidic channel. The processing includes three repeating steps to all the captured frames with the flowing cells: 1) temporal-differencing based flowing cell detection [156, 157]; 2) single-frame ELM-SR based cell type recognition [144]; and 3) cell counting of each type.

6.3.1 Temporal-differencing based Flowing Cell Detection

All the flowing cells in each LR frame need to be detected first. This is realized by the temporal-differencing based background subtraction [156, 157]. Starting from the first two frames, where the first one is the reference (or background) frame and the second is the current (or foreground) frame, moving cell contours in current frame is detected by subtracting it with its previous reference frame to obtain a pixel-by-pixel intensity difference. After subtraction, the regions where the intensity differences are zero indicate no moving cells; and those non-zero difference regions are caused by the motion of cells in the channel, or by the addition and removal of a cell from the sensor field-of-view (FOV). A suitable intensity threshold can be set to identify the contours of moving cells from the background in all frames [156]. The time-difference between

each two consecutive frames is determined by the sensor frame rate. Note that each detected cell in one frame will be assigned with one unique identification number, which means the cell count of the current frame.

6.3.2 ELM-SR based Flowing Cell Recognition

As the raw detected cell images have low resolution, SR processing needs to be performed for better cell type recognition and further counting. In order to resolve the problems of low flow speed and large storage requirement, the inherent limitations of the previous microfluidic imaging system with multi-frame SR processing [44, 63], the machine-learning based single-frame SR is developed for the proposed contact-imaging microfluidic cytometer.

The single-frame SR usually includes two classes, 1) interpolation and sharpening based [72]; and 2) example pattern based [145, 146]. The first one uses generic image interpolation techniques such as bilinear and bicubic interpolation, which cannot recover HF components from LR image, while the second one can extract new HF information. The machine learning based approach that explores the pattern of training images to recover SR images falls into the second class.

Figure 6-10 Structure of the extreme learning machine model.

Among numerous machine learning algorithms, neural-network based one shows low complexity. Particularly, the ELM, which was developed for single-hidden-layer feed forward neural networks (SLFNs), has only one input layer, one hidden layer and one output layer as shown in Figure 6-10. It has a major merit of randomly generated weights between input layer and hidden layer, making it tuning-free without expensive iterative training process [144]. This advantage over other machine learning approaches such as support vector machine [158] and back propagation [159] makes it suitable for SR processing and recognition error recovery in microfluidic lensless imaging, since the number of training cell images can be large if one needs to cover all different cell types under different appearances.

6.3.2.1 ELM-SR Training and Testing

As shown in Figure 6-11(A), the ELM-SR includes off-line training and on-line testing. In the training step, a reference model is trained that can map the interpolated LR images with the HF components extracted from the HR images from the training library. The off-line HR training image library is first generated by taking the grayscale HR images of cells with an inverted microscope camera (Olympus IX71, Tokyo, Japan). For one type of cell to generate a HR library, the cell solution is prepared and dropped into the inlet of one microfluidic channel that is bonded on a cover glass. This helps mimic the environment of the microfluidic channel bonded on the CMOS image sensor. Cells suspended in the channel can have different rotations or details in appearance. Thus, to ensure a more complete library, a large number of typical images are taken to generate an HR image library for one cell type under different appearances. Thereby, the trained reference model is more generic (as a cell neuron) when used for the on-line SR recovery. Note that this work is towards automatic cell counting such that we assume that the cell types in the sample solution are known or to be pre-characterized in advance. If a new pattern appears, we need to train a new ELM-SR model, which is off-line.

In the off-line training step, given the input of HR image $HR_{M \times N}$, where M is the row pixel number and N is the column pixel number, a corresponding LR image $LR_{m \times n}$ is first generated through bicubic down sampling as shown in Figure 6-11. Note that the down sampling factor is the same as the SR enhancement factor t , i.e., $M=m \times t$,

$N=n \times t$. Next, the generated LR image $LR_{m \times n}$ is interpolated back to $LR_Int_{M \times N}$, which has the same size of $HR_{M \times N}$ but blurred and lack of HF component details. As such, by subtracting the HR image $HR_{M \times N}$ with the interpolated LR image $LR_Int_{M \times N}$, the HF component $HF_{M \times N}$ is obtained, i.e.,

$$HF_{M \times N} = HR_{M \times N} - LR_{M \times N}. \quad (6-4)$$

Based on p HF images $HF_{M \times N}$ from the training library, the training targeting value T is obtained which is a $p \cdot MN \times I$ row vector of all the pixels intensity values in HF images. Meanwhile, the pixel intensity pattern existed in $LR_Int_{M \times N}$ is extracted by a 3×3 pixel patch $P(i, j)$ centered at pixel (i, j) of $LR_Int_{M \times N}$ to search through the whole image, where $1 \leq i \leq M-1$ and $1 \leq j \leq N-1$. As such, the column vectors extracted from all patches in p interpolated images $LR_Int_{M \times N}$ compose the feature matrix X . Thus, the ELM training dataset (X, T) is generated.

As such, ELM can take the input (X, T) to ELM to calculate a row vector β containing the weights by

$$T = \beta G(AX + B) \quad (6-5)$$

where G is a sigmoid function, and A and B are randomly generated matrix [144]. The training data with A , B and β can be used for the ELM-SR reference model.

In the on-line testing step, when a detected LR cell image $LR'_{m \times n}$ is inputted, the corresponding SR image can be recovered using the same A , B and the trained β as follows. The resolution of $LR'_{m \times n}$ is first enhanced by t times through bicubic interpolation to $LR_Int'_{M \times N}$. The same patch searching method used in the ELM-SR training is applied to extract the feature matrix X' from $LR_Int'_{M \times N}$. Thus, one can calculate the row vector T' that includes the recovered HF components $HF'_{M \times N}$ for the input LR image $LR'_{m \times n}$. As such, the final SR image $SR'_{M \times N}$ is recovered with the sufficient HF details for cell type recognition by

$$SR'_{M \times N} = HF'_{M \times N} + LR_Int'_{M \times N}. \quad (6-6)$$

Figure 6-11 ELM-SR processing flowchart. The training is performed off-line to generate a reference model that can map the interpolated LR images with the HF components from the HR images; and the testing is performed on-line to recover a SR image from the input LR image with the reference model.

6.3.2.2 Flowing Cell Recognition

Cell type recognition in the developed microfluidic cytometer is performed after recovering the SR image $SR'_{M \times N}$ from the input LR image $LR'_{m \times n}$. The recognition process is shown in Figure 6-12. Assume that the samples of interest include two types of cells, two reference models need to be trained for each type of cell. Then when a detected LR cell $LR'_{m \times n}$ is inputted, two SR images, $SR1'_{M \times N}$ and $SR2'_{M \times N}$ can be recovered, each corresponding to one reference model. Afterwards, $SR1'_{M \times N}$ and $SR2'_{M \times N}$ are compared with the typical HR images $HR1_{M \times N}$ and $HR2_{M \times N}$ in the training libraries, where the mean structural similarity (MSSIM/SSIM) index [148] is employed to characterize the similarity. The SSIM is a full reference metric between 0 and 1 to indicate the similarity between one SR image with one distortion-free reference HR image by

$$SSIM(SR, HR) = \frac{(2\mu_{SR}\mu_{HR})(2\sigma_{SR,HR})}{(\mu_{SR}^2 + \mu_{HR}^2)(\sigma_{SR}^2 + \sigma_{HR}^2)}, \quad (6-7)$$

where μ_{SR} and μ_{HR} are the means of the SR and HR images, σ_{SR}^2 and σ_{HR}^2 are the variances of the SR and HR images, and $\sigma_{SR,HR}$ is the covariance of the SR and HR images. It is proven to be consistent with human eye perception compared with traditional metric such as peak signal-to-noise ratio (PSNR) and mean squared error (MSE) [148]. The MSSIM is the average of the SSIMs for one SR image with all the typical HR images,

$$MSSIM(SR, HRlib) = \frac{1}{K} \sum_{k=1}^K SSIM(SR, HR_k) \quad (6-8)$$

where K is the number of typical HR images in the HR training library. For $SR1'_{M \times N}$ and $SR2'_{M \times N}$, MSSIM1 and MSSIM2 can be calculated. Then we categorize the cell to the type that has the stronger MSSIM.

Figure 6-12 Flowing cell recognition flowchart. The detected LR image is processed with ELM-SR to obtain SR images according to different off-line trained models. Then,

the SR images are compared with typical HR cell images in the library with cell categorized to one type that has the largest MSSIM.

As such, with the ELM-based single-frame SR processing, the developed microfluidic cytometer can have much better imaging capability to distinguish cell details in the continuously flowing microfluidic channel.

6.3.3 Flowing Cell Counting

After recognizing the type for all the detected cells flowing through the channel, the total number of each cell type in the sample of interest can be enumerated. For one cell type, as the cell number in each frame was already known after the temporal-differencing based cell detection step, we subtracted the cell number of the current frame with its previous reference frame to obtain a difference value. If this difference was larger than zero, meaning that new cells have flown into the sensor FOV to increase the cell count over the previous frame. As such, we added this difference to the total cell count. By adding all the positive differences after processing the whole series of frames, the total number for one cell type is obtained. For other cell types, the counting procedure is processed in the same and hence their concentration ratio can be eventually obtained.

As such, the detection, recognition, and counting for all the flowing cell types in the testing sample can be achieved, realizing the function of the contact-imaging based microfluidic cytometer.

6.4 Measurement Results

To evaluate the accuracy of the developed contact-imaging microfluidic cytometer with machine-learning for single-frame super-resolution processing, both of the microbead solution and mixed RBC and tumor cell solution were tested with measurement results compared with a commercially available flow cytometer (BD Accuri C6, NJ, US). Before the system characterization, the design CMOS image sensor chip is tested and the measurement results are presented.

6.4.1 CMOS Image Sensor Chip

Based on the aforementioned system specifications, one high speed SR CMOS image sensor is designed and fabricated with GlobalFoundries (GF) 0.18 μm 1P6M mixed-signal CMOS process. The photo of the chip is shown in Figure 6-13, and the characteristics of the CMOS image sensor are summarized in Table 6-1.

Figure 6-13 Chip photo of the taped out CMOS image sensor with GF standard CMOS 0.18 μm process.

The total chip area is 2.5mm $\times$ 5.0mm, where the pixel array size is 1.28mm $\times$ 1.28mm, column readout occupies 1.28mm $\times$ 1.6mm, and the remaining is for digital core implementation where the on-chip super-resolution processing block only needs area of 0.16mm $\times$ 1.4mm. A large clock bus with group buffers employed to handle a high frequency of 800MHz clock, which is the speed of the up-down counter in the ADC.

Table 6-1 Summary of characteristics of the super-resolution CMOS image sensor.

Parameter	Value
Technology	GF 0.18 μm CMOS
Supply Voltage	3.3V
Array Size	128(H) $\times$ 128(V)
Pixel Size	10 μm $\times$ 10 μm
Pixel Type	3T-APS
Fill Factor	56%
Die Size	12.5mm ² (2.5mm $\times$ 5.0mm)
Chip Size	144 mm ² (12mm $\times$ 12mm)
ADC Resolution	10-bit
Frame Rate	1750fps

Figure 6-14 Readout timing results by the designed column-parallel CMOS image sensor.

Figure 6-14 shows the readout simulation results of the designed CMOS image sensor. The whole row-readout time is 1.7 μs , where signal V_{sig} readout needs 1.28 μs

and the reset V_{rst} requires $0.32\ \mu\text{s}$. During the row-readout time, the 10-bit single-slope ADC operates at 800MHz clock in order to convert one whole row data within $1.7\ \mu\text{s}$. During the signal readout phase, the pixel output is amplified through column amplifier. The voltage level also moves up to the reference voltage level and is connected to the ADC comparator input. The pixel data of one row is stored in the column-wise SRAM and is then sent to digital processing block. The SRAM readout time can achieve 20ns/column. Therefore, the total readout speed from SRAM output can achieve 1750 frame/s.

We also verified the on-chip single-frame SR image processing algorithm for bio-microfluidic imaging application. Colloid particle such as polystyrene bead is a common method to simulate the cell behavior in bio-microfluidic imaging system (as shown in Figure 6-15. Figure 6-16(a) shows the picture of colloid particle flowing in microfluidic device. The particle has diameter $15.7\ \mu\text{m}$ similar to the size of a cancer cell. The original image of particle (8×8) is processed, shown in Figure 6-16(b), by the on-chip single-frame SR algorithm to generate the processed image of 32×32 . As shown in Figure 6-16(c), when compared to the original picture, the image after super-resolution processing shows more details with 4X improved resolution of the particle. Therefore, a high-speed CMOS image sensor with SR on-chip processing ability is demonstrated for bio-microfluidic contact imaging system towards high-throughput cell detection application. After the circuit level measurement results, the system measurement results for microfluidic cytometer are presented in the following.

Figure 6-15 Bio-microfluidic device that can separate cancer cells out from normal cells by using gravity.

Figure 6-16 Particle image processing results of single-frame SR. (a) particle flowing in microfluidic device. (b) Original 8×8 LR particle image. (c) 32×32 SR image processed by the on-chip single-frame SR processing.

6.4.2 Counting Performance Characterization

Figure 6-17 Comparison of concentration measurement results for $6\mu\text{m}$ microbead solution between the developed microfluidic cytometer and the commercial flow cytometer. The average counting result of the developed microfluidic cytometer matched well with that of the commercial cytometer with 8% error.

As described in the previous section, the $6\mu\text{m}$ polystyrene microbead solution was prepared with a concentration of $100\mu\text{L}^{-1}$ measured by the commercial flow cytometer. The $6\mu\text{m}$ sample was flushed through the microfluidic channel at a flow rate of

5 μ L/min by a syringe pump. Then, a series of 640 frames were captured by the CMOS image sensor for a period of one minute. The total number of microbeads was automatically counted by the developed image processing algorithm. The same process was repeated for 6 minutes, and the measured concentrations of the microbead are shown in Figure 6-17. The final microbead concentration is calculated by averaging the counting results of each group, which was 91 μ L⁻¹ with only 8% error when compared with the result 99 μ L⁻¹ by the commercial flow cytometer.

Figure 6-18 Comparison of counting results of different microbead concentration

solutions between the developed microfluidic cytometer and the commercial flow cytometer. (A) Measurement results correlate well between the developed system and the commercial one ($y=0.97x-8$, correlation coefficient=0.996). (B) The Bland-Altman analysis of the measurement results between the developed one and the commercial one show a mean bias of $-13.6\mu\text{L}^{-1}$, the lower 95% limit of agreement by $-61.0\mu\text{L}^{-1}$, and the upper 95% limit of agreement by $33.8\mu\text{L}^{-1}$.

To further evaluate the developed microfluidic cytometer, five microbead samples of different concentrations, ranging from $\sim 50\mu\text{L}^{-1}$ to $\sim 800\mu\text{L}^{-1}$ were prepared. The flow rate and imaging time were used under the same settings. As shown from Figure 6-18(A), the measurement results of the developed microfluidic cytometer correlated well with the commercial flow cytometer with a correlation coefficient of 0.99. Moreover, in order to assess the agreement between the two methods, the Bland-Altman analysis was also performed. As the results shown in Figure 6-18(B), a systematic mean bias of $-13\text{cells } \mu\text{L}^{-1}$ was obtained for the developed microfluidic cytometer compared with the commercial flow cytometer. The under counting performance was due to the dead volume in the channel inlet/outlet as well as the cell lost and sedimentation in the tubing.

6.4.3 Off-line ELM-SR Reference

For the cell recognition, HepG2 and RBC cells were used. The resolution enhancement factor of X4 was used to improve the LR images. Larger enhancement factor can be selected but at the expense of longer processing time and complexity. Since current LR pixel is $2.2\mu\text{m}$, after SR processing the equivalent pixel size is reduced to 550nm , enough for the normal cell diagnosis.

The off-line training HR image library of HepG2 and RBC was first built. The raw HR images of HepG2 and RBC were taken by the microscope camera at X40 objective, and saved into the HR image library with the size of 48×48 , as shown in Figure 6-19(A1-A4). Then, the corresponding 12×12 LR images were obtained by bicubic down sampling the HR images as shown in Figure 6-19(B1-B4). Next, these LR cell images were interpolated back to the same size of their original HR images, i.e., 48×48 . Note that the detailed structures cannot be observed from the interpolated images

because the interpolation cannot recover the HF components, as shown in Figure 6-19(C1-C4).

Figure 6-19 ELM-SR off-line training images for HepG2 and RBC cells. (A) The original HR images for HepG2 cell with two different appearances; and the same for RBC cells. (B) The corresponding LR images. (C) The interpolated images of LR images, which cannot show HF details. (D) The extracted HF components. The scale bar indicates $5\mu\text{m}$.

After that, the HF components for each training cell image were obtained by subtracting the interpolated cell images from the original HR images, such as Figure 6-19(D1-D4). As such, the training library was generated and inputted to perform the ELM-SR training and also obtain the reference model (A , B and β). For the current mixed HepG2 and RBC samples, there are 30 HR images selected for each cell type to build the training library.

6.4.4 On-line ELM-SR Recognition and Counting

Figure 6-20 ELM-SR on-line testing results for HepG2 and RBC cells. The resolution is improved by 4X after ELM-SR processing. (A) The HepG2 on-line testing image and the recovered SR image. (B) The RBC on-line testing image and the recovered SR image. (C) The comparison of MSSIM for different SR images obtained under different training models. The detected HepG2 and RBC can be correctly categorized to its type as the SR image recovered by corresponding ELM-SR model produces a larger MSSIM when compared to each cell HR library. The scale bar indicates 5 μm .

After building the off-line training image library and ELM-SR reference models of HepG2 and RBC cells, the on-line ELM-SR processing was performed when an LR image of HepG2 or RBC cell was captured as shown in Figure 6-20(A1) and (B1). The

recovered SR images using the corresponding trained ELM-SR models are shown in Figure 6-20(A4) and (B4), which are defined as HepG2 $SR_{Hep-Model}$ and RBC $SR_{RBC-Model}$. It can be clearly observed that the ELM-SR recovered images show much better cell internal and edge information that the interpolated images of Figure 6-20(A3) and (B3) cannot show. The biconcave shape of the SR image of RBC cell can also be observed with sufficient difference from the HepG2 cell.

In addition, the SR image of HepG2 cell recovered by the RBC trained model (HepG2 $SR_{RBC-Model}$) and the SR image of RBC SR cell recovered by the HepG2 trained model (RBC $SR_{Hep-Model}$) are also shown in Figure 6-20(A5) and Figure 6-20(B5). One can notice large differences when compared with the original HR images. The MSSIMs with HepG2 library and RBC library are shown in Figure 6-20(C). The MSSIMs for HepG2 $SR_{Hep-Model}$ and RBC $SR_{RBC-Model}$ with the HR HepG2 and RBC image libraries are 0.5190 and 0.7608, respectively; and the MSSIMs for HepG2 $SR_{RBC-Model}$ and RBC $SR_{Hep-Model}$ are 0.1554 and 0.2378, respectively. The difference $\Delta MSSIM$ of 0.3636 and 0.5230 indicate that the SR image of both HepG2 and RBC have enough MSSIM difference to be distinguished.

Figure 6-21 Commercial flow cytometer counting results for the mixed RBC and HepG2 cells. The absolute counts of RBC and HepG2 are 1054 and 978 with the ratio of RBC/HepG2 by $51.9\%:48.1\% = 1.08:1$.

Furthermore, the ELM-SR was applied to distinguish different flowing cell types when the cell count of each type can be obtained. The ratio between RBC and HepG2 cells was prepared and measured by the commercial flow cytometer with the ratio of 1.08:1 (51.9% : 48.1%) as indicated in Figure 6-21. Then, the sample was tested using the developed microfluidic cytometer at a flow rate of 5 $\mu\text{L}/\text{min}$. As shown in Table 6-2, the sample was tested for 6 groups, each group for one minute. The mean RBC/HepG2 ratio is 52.60%:47.40% = 1.11:1 with the coefficients of variation (CV) of 0.10, which matched well with the commercial flow cytometer result (1.08:1). The CV is lower than many other reported microfluidic cytometers (>15%) [160]. Based on the current sample concentration, the average throughput was 3080 min^{-1} . Although the throughput is relatively low from the commercial flow cytometry standard, it can be further improved by increasing the sample concentration and flow rate. Moreover, the continuous microfluidic flow developed in this work can enable larger volume of sample solution to be examined in each test when compared with the drop and flow method in [44, 63].

Table 6-2 Measured RBC and HepG2 counting results of the developed microfluidic cytometer with ELM-SR based cell recognition.

Group	RBC (# μL^{-1})	HepG2 (# μL^{-1})	RBC/HepG2
1	239 (54.32%)	201 (45.68%)	1.19
2	338 (50.22%)	335 (49.78%)	1.01
3	260 (53.72%)	224 (46.28%)	1.06
4	435 (52.98%)	386 (47.02%)	1.12
5	340 (55.74%)	270 (44.26%)	1.26
6	334 (49.85%)	336 (50.15%)	0.99
Mean	324 (52.60%)	292 (47.40%)	1.11
Stdev	70	72	0.11
CV	0.22	0.25	0.10

6.5 Conclusions

With the use of extreme learning machine for single-frame super-resolution processing, one prototype of contact-imaging based microfluidic cytometer is demonstrated for cell recognition and counting. The developed system resolves the resolution limitation of contact imaging by on-line image recognition based super-resolution processing, which enables continuous high throughput flowing cell recognition and counting. The developed system is validated with comparison to the commercial flow cytometer. The measured results show that the developed system can reach less than 8% error for counting absolute number of microbeads, and can also recognize cell ratio by 0.10 coefficient of variation for the RBC and HepG2 cells in a mixed solution.

The biological cells are suspended in the fluid when they are flowing by the CMOS sensor. In addition, there is a 6 μm thick PDMS layer coated on the sensor for chip bonding. As such, contact imaging in this work thereby just emphasizes a close distance between the cell and the CMOS sensor as compared to the conventional one using optical microscopy. It is always desirable to minimize the distance between the cells and the sensor in order to improve the image contrast as well as resolution [43, 62]. However, the physical distance is the fundamental limitation of one contact imaging system with poor resolution. Therefore, considering this first-priority limitation, we have developed an ELM-SR based SR method to recover the low resolution of the contact images of cells.

Furthermore, different from the commercial flow cytometry that can measure FSC and SSC signals, our contact-imaging based cytometer has only one photo detector at the bottom, i.e., the CMOS image sensor. Thus, it can only capture the projected images with light source illuminating from above, similar to the FSC. Meanwhile, the illumination light beams can be arranged with different angles of incidence [161]. When the angle of incidence increases to 90° , the projected images on the CMOS sensor will be equivalent to SSC. Such a design would furnish another strong capability of the proposed contact-imaging based cytometer.

In addition, as for the choice of samples, RBCs and HepG2 tumor cells are among the most common cell types that commercial flow cytometers or other cell counting systems usually deal with. As a preliminary study, we used our prototype to analyse

and categorize these two common types of cells into their respective groups according to the notably improved image resolution, which cannot be achieved by the conventional on-chip contact imaging system [62]. In the future follow-up studies, we will further improve this platform on different cell groups with more delicate differences in size and other cellular properties.

To clarify the contribution in this work, for the CMOS image sensor design, I mainly worked on the column-parallel readout circuit design, the top level verification and integration with on-chip SR processing block, and the FPGA-based testing system with MATLAB-GUI based timing control. In building the microfluidic flow cytometer prototype, I designed and fabricated the microfluidic channel, and integrated it with CMOS image sensor, and built the small PCB hardware for the sensor. I proposed and realized the ELM-SR processing. I realized all the image processing algorithms. I also performed all the bio-sample preparation and testing, analyzed the data and wrapped up the results with paper writing.

Chapter 7

Conclusions and Future Research

7.1 Conclusions

Towards the personalized bio-imaging diagnosis in current aging society, miniaturized bio-instrument with non-invasive sensing capability is demanded, where the conventional bulky optical lens and other mechanical components are no longer applicable. As such, the LOC systems that integrate microfluidics, MEMS and CMOS sensors for bio-imaging diagnosis are critically needed, which further lead to the requirement of multimodal contact imaging. Towards this end, we have studied CMOS multimodal sensors for LOC integrated bio-imaging diagnosis systems in this thesis, including: 1) CMOS CMUT sensor for ultrasound imaging; 2) CMOS ISFET sensor for ion imaging; and 3) CMOS optical sensor for microfluidic contact imaging.

Firstly, for CMOS CMUT ultrasound sensor, a two-channel AFE IC consisting of a high-frequency preamplifier and two pulsers is demonstrated to integrate with high frequency CMUT array. The pulse can drive HV pulses from 0 to 30V at 43pF load and 16.2ns delay, and the preamplifier achieves over 60dB Ω transimpedance gain with 75MHz bandwidth. A successful pulse-echo acoustic testing using the developed AFE IC with the CMUT device in an oil-immersed environment is verified, which lays the foundation for the three-dimensional high resolution imaging for non-invasive glaucoma diagnosis. Secondly, the dual-mode ion sensor integrates the ISFET with standard 4T-CMOS image sensor (CIS) to realize pH sensing from chemical domain as well as image sensing from optical domain. Measurement results show a sensitivity of 103.8mV/pH and FPN reduction from 4% to 0.3% with a readout speed of 1200fps. A high accuracy optical/pH imaging for contact microbead is demonstrated by pruning sensed data with references from multi-domains, which is targeting for high accuracy DNA sequencing. Thirdly, a microfluidic contact imaging system has been developed with PDMS microfluidic channel integrated on top of CMOS image sensor for the application of flowing cell detection, recognition and counting. To resolve the raw spatial resolution limitation from pixel size, the ELM-SR is proposed to recover high-

frequency loss in detected cell contacting images. Less than 8% counting error and 0.10 coefficient of variation for cell-ratio measurement is demonstrated compared with commercial flow cytometry.

The developed CMOS multimodal sensor based LOC systems have shown great potential to provide a global e-healthcare solution in the coming aging society. Through the continuing advancement of miniaturized diagnostic technology, future clinicians and patients will have access to more accurate, timelier, and far less expensive diagnosis of disease.

7.2 Recommendations for Further Work

Based on above works, there are three recommended future works for this thesis.

The first recommended work is to develop and implement beamforming and further digital image processing to realize the real ultrasound imaging function. Then the in vivo testing for the glaucoma diagnosis of the aging population can be carried on. Future designs can also include more ultrasound blocks such as TGC, ADC, on-chip control and processing, etc.

The second recommended work is to further integrate the dual-mode CMOS ISFET sensor with microwell array to perform the actual DNA sequencing testing. The sensing area partition between optical sensing and chemical sensing for optimized sensitivity also needs to be studied.

The third recommended work is to develop large pixel arrayed CMOS image sensor with on-chip cell detection algorithms so that we can integrate microfluidics with our custom designed image sensor. Then, the off-chip processing can be replaced and the throughput can be improved. For the current prototype, the integration of on-chip pump also needs to be explored. A chip socket can be built on PCB so that if people want to change a new chip, they can easily plug it in/out without iron soldering. We can also change another sensor chip with larger die area so that more parallel channels can be built on it to increase throughput. All these work will finally lead to a portable cell imaging and flow cytometry system that can be actually used at the point-of-care.

Author's Publication

[Book/Chapter]

- B1. Hao Yu, and Xiwei Huang, “3D IC Design Automation Considering Dynamic Power and Thermal Integrity,” Chapter in Three-Dimensional Integration of Integrated Circuits, C. S. Tan, K.-N. Chen, and S. J. Koester (Editors), Pan Stanford Publishing, 2011.

[Journal]

- J10. Xiwei Huang, Jinhong Guo, Xiaolong Wang, Mei Yan, Yuejun Kang, and Hao Yu, “A Contact-imaging based Microfluidic Cytometer with Machine-learning for Single-frame Super-resolution Processing,” *PLoS ONE*, vol. 9, no.8, pp. e104539, 2014.
- J9. Xiwei Huang, Xiaolong Wang, Mei Yan, and Hao Yu, “A Robust Recognition Error Recovery for Micro-flow Cytometer by Machine-learning Enhanced Single-frame Super-resolution Processing,” *Integration, the VLSI Journal*, 2014. (DOI: 10.1016/j.vlsi.2014.07.004)
- J8. Jinhong Guo, Xiwei Huang, Dongyuan Shi, Hao Yu, Ye Ai, Changming Li, and Yuejun Kang, “Portable Resistive Pulse-Activated Lens-Free Cell Imaging System,” *RSC Advances*, vol. 4, pp. 56342-56345, 2014.
- J7. Jinhong Guo, Liang Chen, Xiwei Huang, Chang Ming Li, Ye Ai, and Yuejun Kang, “Dual Characterization of Biological Cells by Optofluidic Microscope and Resistive Pulse Sensor,” *Electrophoresis*, 2014. (DOI: 10.1002/elps.201400268)
- J6. Xiwei Huang, Xu Liu, Mei Yan, Dongping Wu, and Hao Yu, “A Dual-mode Large-arrayed CMOS ISFET Sensor for Accurate and High-throughput pH Sensing in Biomedical Diagnosis,” *IEEE Transactions on Biomedical Engineering (TBME)*, 2014. (under review)
- J5. Jinhong Guo, Xiwei Huang, Dongyuan Shi, Ye Ai, Hao Yu, and Yuejun Kang, “Computer-aided Design of A Microfluidic Sensor for Tumor Cell Detection and Enumeration,” *Electrophoresis*, 2014. (accepted with revision)
- J4. Xiwei Huang, Hao Yu, and Mei Yan, “A Single-frame Super-resolution CMOS

Image Sensor for Portable Lensless Microfluidic Imaging System,” *Biosensors*, 2014. (under submission)

- J3. Hyouk-Kyu Cha, Dongning Zhao, **Xiwei Huang**, Jia Hao Cheong, Bin Guo, Hongbin Yu, Hao Yu, and Minkyu Je, “A High-Voltage High-Frequency CMOS Analog Front-End IC for Medical Ultrasound Imaging,” *Taylor & Francis International Journal of Electronics*, 2014. (under review)
- J2. **Xiwei Huang**, Chun Zhang, Hao Yu, and Wei Zhang, “A Nano-Electro-Mechanical-Switch based Thermal Management for 3D Integrated Many-core Memory-Processor System,” *IEEE Transactions on Nanotechnology (TNANO)*, vol.11, no.3, pp. 588-600, May 2012.
- J1. Hanhua Qian, **Xiwei Huang**, Hao Yu, and Chip Hong Chang, “Cyber-physical Thermal Management of 3D Multi-core Cache-Processor System with Microfluidic Cooling,” *Journal of Low Power Electronics (JOLPE)*, vol. 7, no. 1, pp. 110-121, 2011.

[Conference]

- C11. **Xiwei Huang**, Jing Guo, Mei Yan, and Hao Yu, "A 64×64 1200fps Dual-mode CMOS Ion-Image Sensor for Accurate DNA Sequencing," IEEE/ACM Asia and South Pacific Design Automation Conference - University Design Contest (ASP-DAC UDC), January 2015.
- C10. **Xiwei Huang**, Xiaolong Wang, Mei Yan, and Hao Yu, “Machine-learning based Single-frame Super-resolution Processing for Microfluidic Contact Imaging,” International Symposium on Microchemistry and Microsystems (ISMM), August 2014.
- C9. **Xiwei Huang**, Fei Wang, Jing Guo, Mei Yan, Hao Yu, and Kiat Seng Yeo, “A 64×64 1200fps CMOS Ion-Image Sensor with Suppressed Fixed-Pattern-Noise for Accurate High-throughput DNA Sequencing,” *IEEE Symposium on VLSI Circuits (VLSIC)*, June 2014.
- C8. **Xiwei Huang**, Mei Yan, Hao Yu, and Kiat Seng Yeo, “Design of Dual-mode CMOS ISFET Sensor for DNA Sequencing,” IEEE International Solid-State Circuits Conference (ISSCC) Student Research Preview, February 2014.
- C7. **Xiwei Huang**, Jinhong Guo, Mei Yan, Ramakrishna Kakarala, Ningmei Yu,

- Yuejun Kang, and Hao Yu, “A Real-time Lensless Optofluidic Imaging System for Flowing Cell Counting,” International Conference on Optofluidics, August, 2013.
- C6. Xiwei Huang, Jia Hao Cheong, Hyouk-Kyu Cha, Hongbin Yu, Minkyu Je, and Hao Yu, “A High-frequency Transimpedance Amplifier for CMOS Integrated 2D CMUT Array towards 3D Ultrasound Imaging,” International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), July 2013.
- C5. Tongxi Wang, Xiwei Huang, Mei Yan, Hao Yu, Kiat Seng Yeo, Ismail Cevik, and Suat Ay, “A 6 μ W Ultra-low Power CMOS Image Sensor for Biomedical Applications,” IEEE Asia Pacific Conference on Circuits and Systems (APCCAS) (Special Session), December 2012.
- C4. Tongxi Wang, Xiwei Huang, Qixiang Jia, Mei Yan, Hao Yu, and Kiat Seng Yeo, “A Super-resolution CMOS Imager for Microfluidic Imaging Applications,” IEEE Biomedical Circuits and Systems Conference (BIOCAS), November 2012.
- C3. Mei Yan, Xiwei Huang, Qixiang Jia, Revanth Nadipalli, Yang Shang, Hao Yu, Minkyu Je and Kiat Seng Yeo, “High-speed CMOS Image Sensor for High-throughput Lensless Microfluidic Imaging System with Point-of-care Application,” SPIE Electronic Imaging, February 2012.
- C2. Xiwei Huang, Hao Yu, and Wei Zhang, “NEMS based Thermal Management for 3D Many-core System,” ACM/IEEE International Symposium on Nanoscale Architectures (NANOARCH), June 2011.
- C1. Hanhua Qian, Xiwei Huang, Hao Yu, and Chip Hong Chang, “Real-time Thermal Management of 3D Multi-core System with Fine-grained Cooling Control,” IEEE International 3D System Integration Conference (3DIC), November 2010.

Bibliography

- [1] WHO. "Global Brief for World Health Day 2012," http://www.who.int/ageing/publications/whd2012_global_brief/en/.
- [2] WHO. "Ageing and Life Course," <http://www.who.int/ageing/en/>.
- [3] T. Wilson, "Confocal microscopy," *Academic Press: London, etc*, vol. 426, pp. 1-64, 1990.
- [4] X. Huang, J. H. Cheong, H.-K. Cha, H. Yu, M. Je, and H. Yu, "A high-frequency transimpedance amplifier for CMOS integrated 2D CMUT array towards 3D ultrasound imaging," International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), pp. 101-104, 2013.
- [5] I. Kim, H. Kim, F. Griggio, R. L. Tutwiler, T. N. Jackson, S. Trolier-McKinstry *et al.*, "CMOS ultrasound transceiver chip for high-resolution ultrasonic imaging systems," *IEEE Transactions on Biomedical Circuits and Systems (TBCAS)*, vol. 3, no. 5, pp. 293-303, 2009.
- [6] G. Gurun, P. Hasler, and F. L. Degertekin, "Front-end receiver electronics for high-frequency monolithic CMUT-on-CMOS imaging arrays," *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 58, no. 8, pp. 1658-1668, 2011.
- [7] H. M. Shapiro, *Practical Flow Cytometry*, 4th ed.: John Wiley & Sons, 2003.
- [8] A. L. Givan, *Flow Cytometry: First Principles*, 2nd ed.: John Wiley & Sons, 2001.
- [9] M. Brown, and C. Wittwer, "Flow Cytometry: Principles and Clinical Applications in Hematology," *Clinical Chemistry* vol. 46, no. 8, pp. 1221-1229, 2000.
- [10] J. Shendure, and H. Ji, "Next-generation DNA sequencing," *Nature Biotechnology*, vol. 26, pp. 1135-1145, 2008.
- [11] M. Margulies, M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bembien *et al.*, "Genome sequencing in microfabricated high-density picolitre reactors," *Nature*, vol. 437, pp. 376-380, 2005.
- [12] D. R. Bentley, S. Balasubramanian, H. P. Swerdlow, G. P. Smith, J. Milton, C. G. Brown *et al.*, "Accurate whole human genome sequencing using reversible terminator chemistry," *Nature*, vol. 456, pp. 53-59, 2008.
- [13] C. F. Gilks, S. Crowley, R. Ekpini, S. Gove, J. Perriens, Y. Souteyrand *et al.*, "The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings," *The Lancet*, vol. 368, no. 9534, pp. 505-510, 2006.
- [14] S. J. Moon, H. O. Keles, A. Ozcan, A. Khademhosseini, E. Hægstrom, D. Kuritzkes *et al.*, "Integrating microfluidics and lensless imaging for point-of-care testing," *Biosensors and Bioelectronics*, vol. 24, no. 11, pp. 3208-3214, 2009.
- [15] G. J. Kost, *Principles and Practice of Point-of-care Testing*: Lippincott Williams & Wilkins Philadelphia, 2002.

-
- [16] S. K. Sia, and L. J. Kricka, "Microfluidics and point-of-care testing," *Lab on a Chip*, vol. 8, no. 12, pp. 1982-1983, 2008.
 - [17] P. Yager, G. J. Domingo, and J. Gerdes, "Point-of-care diagnostics for global health," *Annual Review of Biomedical Engineering*, vol. 10, pp. 107-144, 2008.
 - [18] B. Korte, *NIH Fact Sheet: Point-of-Care Diagnostic Testing*, 2010.
 - [19] C. D. Chin, V. Linder, and S. K. Sia, "Lab-on-a-chip devices for global health: past studies and future opportunities," *Lab on a Chip*, vol. 7, no. 1, pp. 41-57, 2006.
 - [20] P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam *et al.*, "Microfluidic diagnostic technologies for global public health," *Nature*, vol. 442, pp. 412-418, 2006.
 - [21] G. M. Whitesides, "The origins and the future of microfluidics," *Nature* vol. 442, pp. 368-373, 2006.
 - [22] S. Haeberle, and R. Zengerle, "Microfluidic platforms for lab-on-a-chip applications," *Lab on a Chip*, vol. 7, no. 9, pp. 1094-1110, 2007.
 - [23] D. Figeys, and D. Pinto, "Lab-on-a-Chip: A Revolution in Biological and Medical Sciences," *Analytical Chemistry*, vol. 72, no. 9, pp. 330-335, 2000.
 - [24] Y.-S. Lin, D. Sylvester, and D. Blaauw, "An ultra low power 1V, 220nW temperature sensor for passive wireless applications," *IEEE Custom Integrated Circuits Conference (CICC)*, pp. 507-510, 2008.
 - [25] S. Hanson, and D. Sylvester, "A 0.45–0.7 V sub-microwatt CMOS image sensor for ultra-low power applications," *IEEE Symposium on VLSI Circuits (VLSIC)*, pp. 176-177, 2009.
 - [26] Y.-S. Lin, D. M. Sylvester, and D. T. Blaauw, "A 150pW program-and-hold timer for ultra-low-power sensor platforms," *IEEE International Solid-State Circuits Conference (ISSCC)*, pp. 326-327, 2009.
 - [27] S. Hanson, Z. Foo, D. Blaauw, and D. Sylvester, "A 0.5 V sub-microwatt CMOS image sensor with pulse-width modulation read-out," *IEEE Journal of Solid-State Circuits (JSSC)*, vol. 45, no. 4, pp. 759-767, 2010.
 - [28] G. Ter Haar, "Ultrasound focal beam surgery," *Ultrasound in medicine & biology*, vol. 21, no. 9, pp. 1089-1100, 1995.
 - [29] T. Dada, R. Gadia, A. Sharma, P. Ichhpujani, S. J. Bali, S. Bhartiya *et al.*, "Ultrasound Biomicroscopy in Glaucoma," *Survey of Ophthalmology*, vol. 56, no. 5, pp. 433-450, 2011.
 - [30] A. Assmus, "Early History of X Rays," www.slac.stanford.edu, 1995].
 - [31] D. J. Brenner, and E. J. Hall, "Computed tomography—an increasing source of radiation exposure," *New England Journal of Medicine*, vol. 357, no. 22, pp. 2277-2284, 2007.
 - [32] E. M. Haacke, R. W. Brown, M. R. Thompson, and R. Venkatesan, "Magnetic resonance imaging," *Physical principles and sequence design*, 1999.
 - [33] B. Hu, and M. Nuss, "Imaging with terahertz waves," *Optics letters*, vol. 20, no. 16, pp. 1716-1718, 1995.
-

-
- [34] C. Yang, and D. Psaltis, *Building a Microscopic Microscope*, 2007.
- [35] I. Cicek, A. Bozkurt, and M. Karaman, "Design of a front-end integrated circuit for 3D acoustic imaging using 2D CMUT arrays," *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 52, no. 12, pp. 2235-2241, 2005.
- [36] I. Kim, "Fully Integrated CMOS Ultrasound Transceiver Chip For High-frequency High-resolution Ultrasonic Imaging Systems," The Pennsylvania State University, 2009.
- [37] R. Daw, and J. Finkelstein, "Lab on a chip," *Nature*, vol. 442, no. 7101, pp. 367-367, 2006.
- [38] G. M. Whitesides, "The origins and the future of microfluidics," *Nature*, vol. 442, no. 7101, pp. 368-373, 2006.
- [39] D. Mark, S. Haeberle, G. Roth, F. von Stetten, and R. Zengerle, "Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications," *Chemical Society Reviews*, vol. 39, no. 3, pp. 1153-1182, 2010.
- [40] Y. Zhao, D. Chen, H. Yue, J. B. French, J. Rufo, S. J. Benkovic *et al.*, "Lab-on-a-chip technologies for single-molecule studies," *Lab on a Chip*, vol. 13, no. 12, pp. 2183-2198, 2013.
- [41] M. Medina-Sanchez, S. Miserere, and A. Merkoci, "Nanomaterials and lab-on-a-chip technologies," *Lab on a Chip*, vol. 12, no. 11, pp. 1932-1943, 2012.
- [42] J. Wu, G. Zheng, and L. M. Lee, "Optical imaging techniques in microfluidics and their applications," *Lab on a Chip*, vol. 12, no. 19, pp. 3566-3575, 2012.
- [43] H. Ji, D. Sander, A. Haas, and P. A. Abshire, "Contact imaging: simulation and experiment," *IEEE Transactions on Circuits and Systems I: Regular Papers (TCAS-I)*, vol. 54, no. 8, pp. 1698-1710, 2007.
- [44] G. Zheng, S. A. Lee, S. Yang, and C. Yang, "Sub-pixel resolving optofluidic microscope for on-chip cell imaging," *Lab on a Chip*, vol. 10, no. 22, pp. 3125-3129, 2010.
- [45] D. N. Adamson, D. Mustafi, J. X. J. Zhang, B. Zheng, and R. F. Ismagilov, "Production of arrays of chemically distinct nanolitre plugs via repeated splitting in microfluidic devices," *Lab on a Chip*, vol. 6, no. 9, pp. 1178-1186, 2006.
- [46] T.-W. Huang, and T.-Y. Ho, "A fast routability-and performance-driven droplet routing algorithm for digital microfluidic biochips," *IEEE International Conference on Computer Design (ICCD)*, pp. 445-450, 2009.
- [47] T.-Y. Ho, J. Zeng, and K. Chakrabarty, "Digital microfluidic biochips: A vision for functional diversity and more than Moore," *IEEE International Conference on Computer-Aided Design (ICCAD)*, pp. 578-585, 2010.
- [48] T.-Y. Ho, K. Chakrabarty, and P. Pop, "Digital microfluidic biochips: recent research and emerging challenges," *IEEE International Conference on Hardware/Software Codesign and System Synthesis*, pp. 335-343, 2011.
- [49] Y.-L. Hsieh, T.-Y. Ho, and K. Chakrabarty, "On-chip biochemical sample preparation using digital microfluidics," *IEEE Biomedical Circuits and Systems Conference (BioCAS)*, pp. 297-300, 2011.
-

-
- [50] Y.-L. Hsieh, T.-Y. Ho, and K. Chakrabarty, "A reagent-saving mixing algorithm for preparing multiple-target biochemical samples using digital microfluidics," *IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems (TCAD)*, vol. 31, no. 11, pp. 1656-1669, 2012.
 - [51] Y. Luo, K. Chakrabarty, and T.-Y. Ho, "A cyberphysical synthesis approach for error recovery in digital microfluidic biochips," *Design, Automation & Test in Europe Conference & Exhibition (DATE)*, pp. 1239-1244, 2012.
 - [52] Y. Luo, B. B. Bhattacharya, T.-Y. Ho, and K. Chakrabarty, "Optimization of polymerase chain reaction on a cyberphysical digital microfluidic biochip," *IEEE/ACM International Conference on Computer-Aided Design (ICCAD)*, pp. 622-629, 2013.
 - [53] Y. Luo, K. Chakrabarty, and T.-Y. Ho, "Error recovery in cyberphysical digital microfluidic biochips," *IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems (TCAD)*, vol. 32, no. 1, pp. 59-72, 2013.
 - [54] T. A. Dinh, S. Yamashita, and T.-Y. Ho, "A logic integrated optimal pin-count design for digital microfluidic biochips," *Design, Automation and Test in Europe Conference and Exhibition (DATE)*, pp. 1-6, 2014.
 - [55] T. W. Su, S. Seo, A. Erlinger, and A. Ozcan, "High - throughput lensfree imaging and characterization of a heterogeneous cell solution on a chip," *Biotechnology and bioengineering*, vol. 102, no. 3, pp. 856-868, 2009.
 - [56] S. Seo, T.-W. Su, A. Erlinger, and A. Ozcan, "Multi-color LUCAS: Lensfree on-chip cytometry using tunable monochromatic illumination and digital noise reduction," *Cellular and Molecular Bioengineering*, vol. 1, no. 2-3, pp. 146-156, 2008.
 - [57] S. Seo, T.-W. Su, D. K. Tseng, A. Erlinger, and A. Ozcan, "Lensfree holographic imaging for on-chip cytometry and diagnostics," *Lab on a Chip*, vol. 9, no. 6, pp. 777-787, 2009.
 - [58] L. Repetto, E. Piano, and C. Pontiggia, "Lensless digital holographic microscope with light-emitting diode illumination," *Optics letters*, vol. 29, no. 10, pp. 1132-1134, 2004.
 - [59] U. Gopinathan, G. Pedrini, and W. Osten, "Coherence effects in digital in-line holographic microscopy," *Journal of the Optical Society of America A (JOSA A)*, vol. 25, no. 10, pp. 2459-2466, 2008.
 - [60] X. Cui, L. M. Lee, X. Heng, W. Zhong, P. W. Sternberg, D. Psaltis *et al.*, "Lensless high-resolution on-chip optofluidic microscopes for *Caenorhabditis elegans* and cell imaging," *Proceedings of the National Academy of Sciences*, vol. 105, no. 31, pp. 10670-10675, 2008.
 - [61] X. Heng, D. Erickson, L. R. Baugh, Z. Yaqoob, P. W. Sternberg, D. Psaltis *et al.*, "Optofluidic microscopy—a method for implementing a high resolution optical microscope on a chip," *Lab on a Chip*, vol. 6, no. 10, pp. 1274-1276, 2006.
 - [62] A. Ozcan, and U. Demirci, "Ultra wide-field lens-free monitoring of cells on-chip," *Lab on a Chip*, vol. 8, no. 1, pp. 98-106, 2008.
-

-
- [63] S. A. Lee, R. Leitaο, G. Zheng, S. Yang, A. Rodriguez, and C. Yang, “Color capable sub-pixel resolving optofluidic microscope and its application to blood cell imaging for Malaria diagnosis,” *PloS ONE*, vol. 6, no. 10, pp. e26127, 2011.
 - [64] S. Moon, H. O. Keles, A. Ozcan, A. Khademhosseini, E. Hæggsstrom, D. Kuritzkes *et al.*, “Integrating microfluidics and lensless imaging for point-of-care testing,” *Biosensors and Bioelectronics*, vol. 24, no. 11, pp. 3208-3214, 2009.
 - [65] O. Mudanyali, D. Tseng, C. Oh, S. O. Isikman, I. Sencan, W. Bishara *et al.*, “Compact, light-weight and cost-effective microscope based on lensless incoherent holography for telemedicine applications,” *Lab on a Chip*, vol. 10, no. 11, pp. 1417-1428, 2010.
 - [66] W. Bishara, T. W. Su, A. F. Coskun, and A. Ozcan, “Lensfree on-chip microscopy over a wide field-of-view using pixel super-resolution,” *Optics express*, vol. 18, no. 11, pp. 11181-11191, 2010.
 - [67] P. Georgiou, and C. Toumazou, “An adaptive ISFET chemical imager chip,” IEEE International Symposium on Circuits and Systems (ISCAS), pp. 2078-2081, 2008.
 - [68] J. M. Rothberg, W. Hinz, T. M. Rearick, J. Schultz, W. Mileski, M. Davey *et al.*, “An integrated semiconductor device enabling non-optical genome sequencing,” *Nature*, vol. 475, no. 7356, pp. 348-352, 2011.
 - [69] P. Bergveld, “Development of an Ion-Sensitive Solid-State Device for Neurophysiological Measurements,” *IEEE Transactions on Biomedical Engineering (TBME)*, vol. BME-17, no. 1, pp. 70-71, 1970.
 - [70] J. Bausells, J. Carrabina, A. Errachid, and A. Merlos, “Ion-sensitive field-effect transistors fabricated in a commercial CMOS technology,” *Sensors and Actuators B: Chemical*, vol. 57, no. 1–3, pp. 56-62, 1999.
 - [71] D. L. Hame, L. J. Bousse, J. D. Shott, and J. D. Meindl, “Ion-sensing devices with silicon nitride and borosilicate glass insulators,” *IEEE Transactions on Electron Devices (TED)*, vol. 34, no. 8, pp. 1700-1707, 1987.
 - [72] T. Wang, X. Huang, Q. Jia, M. Yan, H. Yu, and K. S. Yeo, “A super-resolution CMOS image sensor for bio-microfluidic imaging,” IEEE Biomedical Circuits and Systems Conference (BioCAS), pp. 388-391, 2012.
 - [73] T. Wang, X. Huang, M. Yan, H. Yu, K. S. Yeo, I. Cevik *et al.*, “A 96× 96 1V ultra-low power CMOS image sensor for biomedical application,” IEEE Asia Pacific Conference on Circuits and Systems (APCCAS), pp. 13-16, 2012.
 - [74] M. Yan, X. Huang, Q. Jia, R. Nadipalli, T. Wang, Y. Shang *et al.*, “High-speed CMOS image sensor for high-throughput lensless microfluidic imaging system,” IS&T/SPIE Electronic Imaging, pp. 829804-829804-829812, 2012.
 - [75] X. Heng, L. R. Baugh, Z. Yaqoob, P. W. Sternberg, D. Psaltis, and C. Yang, “Optofluidic microscopy—a method for implementing a high resolution optical microscope on a chip,” *Lab on a Chip*, vol. 6, no. 10, pp. 1274-1276, 2006.
 - [76] T. Tokuda, A. Yamamoto, K. Kagawa, M. Nunoshita, and J. Ohta, “A CMOS image sensor with optical and potential dual imaging function for on-chip
-

- bioscientific applications,” *Sensors and Actuators A: Physical*, vol. 125, no. 2, pp. 273-280, 2006.
- [77] T. Tokuda, K. Tanaka, M. Matsuo, K. Kagawa, M. Nunoshita, and J. Ohta, “Optical and electrochemical dual-image CMOS sensor for on-chip biomolecular sensing applications,” *Sensors and Actuators A: Physical*, vol. 135, no. 2, pp. 315-322, 2007.
- [78] J. Matsuo, K. Sawada, H. Takao, and M. Ishida, “Multimodal pH and light imaging devices for dynamic chemical reaction observation,” *International Conference on Miniaturized Systems for Chemistry and Life Sciences (μ TAS)*, pp. 326-328, 2008.
- [79] Y. Xia, and G. M. Whitesides, “Soft lithography,” *Annual review of materials science*, vol. 28, no. 1, pp. 153-184, 1998.
- [80] MICROCHEM, “NANO SU-8 Negative Tone Photoresist Formulations 2-25,” www.microchem.com/pdf/SU8_2-25.pdf.
- [81] E. Stelzer, “Contrast, resolution, pixelation, dynamic range and signal-to-noise ratio: fundamental limits to resolution in fluorescence light microscopy,” *Journal of Microscopy*, vol. 189, no. 1, pp. 15-24, 1998.
- [82] S. U. Ay, “A hybrid CMOS APS pixel for wide-dynamic range imaging applications,” *IEEE International Symposium on Circuits and Systems (ISCAS)*, pp. 1628-1631, 2008.
- [83] E. R. Fossum, “CMOS image sensors: electronic camera-on-a-chip,” *Electron Devices, IEEE Transactions on*, vol. 44, no. 10, pp. 1689-1698, 1997.
- [84] S. U. Ay, “A CMOS Energy Harvesting and Imaging (EHI) Active Pixel Sensor (APS) Imager for Retinal Prosthesis,” *IEEE Transactions on Biomedical Circuits and Systems (TBCAS)*, vol. 5, no. 6, pp. 535-545, 2011.
- [85] T. Chen, P. B. Catrysse, A. El Gamal, and B. A. Wandell, “How small should pixel size be?,” *Electronic Imaging*, pp. 451-459, 2000.
- [86] J. Farrell, F. Xiao, and S. Kavusi, “Resolution and light sensitivity tradeoff with pixel size,” *Electronic Imaging 2006*, pp. 60690N-60698, 2006.
- [87] M. I. Fuller, K. Ranganathan, S. Zhou, T. N. Blalock, J. A. Hossack, and W. F. Walker, “Experimental System Prototype of a Portable, Low-Cost, C-Scan Ultrasound Imaging Device,” *IEEE Transactions on Biomedical Engineering (TBME)*, vol. 55, no. 2, pp. 519-530, 2008.
- [88] A. S. Erguri, H. Yongli, Z. Xuefeng, O. Oralkan, G. G. Yarahoglu, and B. T. Khuri-Yakub, “Capacitive micromachined ultrasonic transducers: fabrication technology,” *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 52, no. 12, pp. 2242-2258, 2005.
- [89] I. Ladabaum, J. Xuecheng, H. T. Soh, A. Atalar, and B. t. Khuri-Yakub, “Surface micromachined capacitive ultrasonic transducers,” *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 45, no. 3, pp. 678-690, 1998.
- [90] H. Yu, B. Guo, K. Haridas, T.-H. Lin, J. Hao Cheong, M. Lin Tsai *et al.*, “Capacitive micromachined ultrasonic transducer based tilt sensing,” *Applied Physics Letters*, vol. 101, no. 15, pp. 153502, 2012.

-
- [91] P. Muralt, N. Ledermann, J. Paborowski, A. Barzegar, S. Gentil, B. Belgacem *et al.*, "Piezoelectric micromachined ultrasonic transducers based on PZT thin films," *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 52, no. 12, pp. 2276-2288, 2005.
 - [92] T. R. Gururaja, "Piezoelectric transducers for medical ultrasonic imaging," *IEEE International Symposium on Applications of Ferroelectrics (ISAF)*, pp. 259-265, 1992.
 - [93] N. Lamberti, G. Caliano, A. Iula, and A. S. Savoia, "A high frequency cMUT probe for ultrasound imaging of fingerprints," *Sensors and Actuators A: Physical*, vol. 172, no. 2, pp. 561-569, 2011.
 - [94] R. O. Guldiken, J. Zahorian, F. Yamaner, and F. Degertekin, "Dual-electrode CMUT with non-uniform membranes for high electromechanical coupling coefficient and high bandwidth operation," *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 56, no. 6, pp. 1270-1276, 2009.
 - [95] P. Kwan Kyu, L. Hyunjoo, M. Kupnik, and B. T. Khuri-Yakub, "Fabrication of capacitive micromachined ultrasonic transducers via local oxidation and direct wafer bonding," *Journal of Microelectromechanical Systems*, vol. 20, no. 1, pp. 95-103, 2011.
 - [96] A. S. Logan, L. L. P. Wong, and J. T. W. Yeow, "A 1-D capacitive micromachined ultrasonic transducer imaging array fabricated with a silicon-nitride-based fusion process," *IEEE/ASME Transactions on Mechatronics*, vol. 16, no. 5, pp. 861-865, 2011.
 - [97] I. O. Wygant, N. Jamal, H. Lee, A. Nikoozadeh, O. Oralkan, M. Karaman *et al.*, "An integrated circuit with transmit beamforming flip-chip bonded to a 2-D CMUT array for 3-D ultrasound imaging," *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 56, no. 10, pp. 2145-2156, 2009.
 - [98] K. K. Park, H. J. Lee, G. G. Yaralioglu, A. S. Ergun, Ö. Oralkan, M. Kupnik *et al.*, "Capacitive micromachined ultrasonic transducers for chemical detection in nitrogen," *Applied Physics Letters*, vol. 91, no. 9, pp. 094102, 2007.
 - [99] S. H. Wong, M. Kupnik, R. D. Watkins, K. Butts-Pauly, and B. T. Khuri-Yakub, "Capacitive micromachined ultrasonic transducers for therapeutic ultrasound applications," *IEEE Transactions on Biomedical Engineering (TBME)*, vol. 57, no. 1, pp. 114-123, 2010.
 - [100] K. K. Shung, J. Cannata, Z. Qifa, and L. Jungwoo, "High frequency ultrasound: A new frontier for ultrasound," *International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, pp. 1953-1955, 2009.
 - [101] I. O. Wygant, Z. Xuefeng, D. T. Yeh, O. Oralkan, A. S. Ergun, M. Karaman *et al.*, "Integration of 2D CMUT arrays with front-end electronics for volumetric ultrasound imaging," *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 55, no. 2, pp. 327-342, 2008.
 - [102] G. I. Athanasopoulos, S. J. Carey, and J. V. Hatfield, "Circuit design and simulation of a transmit beamforming ASIC for high-frequency ultrasonic
-

-
- imaging systems,” *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control (TUFFC)*, vol. 58, no. 7, pp. 1320-1331, 2011.
- [103] Y. Wang, M. Koen, and D. Ma, “Low-noise CMOS TGC amplifier with adaptive gain control for ultrasound imaging receivers,” *IEEE Transactions on Circuits and Systems II: Express Briefs (TCAS-II)*, vol. 58, no. 1, pp. 26-30, 2011.
- [104] M. R. Bosisio, J. M. Hasquenoph, L. Sandrin, P. Laugier, S. L. Bridal, and S. Yon, “Real-time chirp-coded imaging with a programmable ultrasound biomicroscope,” *IEEE Transactions on Biomedical Engineering (TBME)*, vol. 57, no. 3, pp. 654-664, 2010.
- [105] D. Zhao, and H.-K. Cha, “A 30-V transmitter front-end IC for ultrasound medical imaging applications,” *Microelectronics Journal*, vol. 44, no. 3, pp. 185-189, 2013.
- [106] P. Sheng-Yu, M. S. Qureshi, P. E. Hasler, A. Basu, and F. L. Degertekin, “A Charge-Based Low-Power High-SNR Capacitive Sensing Interface Circuit,” *IEEE Transactions on Circuits and Systems I: Regular Papers (TCAS-I)*, vol. 55, no. 7, pp. 1863-1872, 2008.
- [107] L. R. Cenkeramaddi, A. Bozkurt, F. Yalgin Yamaner, and T. Ytterdal, “A low noise capacitive feedback analog front-end for CMUTs in intra vascular ultrasound imaging,” *IEEE Ultrasonics Symposium (IUS)*, pp. 2143-2146, 2007.
- [108] G. Gurun, M. S. Qureshi, M. Balantekin, R. Guldiken, J. Zahorian, P. Sheng-Yu *et al.*, “Front-end CMOS electronics for monolithic integration with CMUT arrays: Circuit design and initial experimental results,” *IEEE Ultrasonics Symposium (IUS)*, pp. 390-393, 2008.
- [109] X. Huang, F. Wang, J. Guo, M. Yan, H. Yu, and K. S. Yeo, “A 64×64 1200fps CMOS Ion-Image Sensor with Suppressed Fixed-Pattern-Noise for Accurate High-throughput DNA Sequencing,” *IEEE Symposium on VLSI Circuits (VLSIC)*, pp. 1-2, 2014.
- [110] C. Graham, and A. M. Hill, “Introduction to DNA Sequencing,” *DNA Sequencing Protocols*, Methods in Molecular Biology™ C. Graham and A. M. Hill, eds., pp. 1-12: Humana Press, 2001.
- [111] J. Prober, G. Trainor, R. Dam, F. Hobbs, C. Robertson, R. Zagursky *et al.*, “A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides,” *Science*, vol. 16 pp. 336-341, 1987.
- [112] P. Bergveld, “Thirty years of ISFETOLOGY: What happened in the past 30 years and what may happen in the next 30 years,” *Sensors and Actuators B: Chemical*, vol. 88, no. 1, pp. 1-20, 2003.
- [113] C. Toumazou, L. M. Shepherd, S. C. Reed, G. I. Chen, A. Patel, D. M. Garner *et al.*, “Simultaneous DNA amplification and detection using a pH-sensing semiconductor system,” *Nat Meth*, vol. 10, no. 7, pp. 641-646, 2013.
- [114] M. J. Milgrew, and D. R. S. Cumming, “Matching the Transconductance Characteristics of CMOS ISFET Arrays by Removing Trapped Charge,” *IEEE Transactions on Electron Devices (TED)*, vol. 55, no. 4, pp. 1074-1079, 2008.
-

-
- [115] C. Z. D. Goh, P. Georgiou, T. G. Constandinou, T. Prodromakis, and C. Toumazou, "A CMOS-Based ISFET chemical imager with auto-calibration capability," *IEEE Sensors Journal*, vol. 11, no. 12, pp. 3253-3260, 2011.
- [116] P. A. Hammond, D. Ali, and D. R. S. Cumming, "Design of a single-chip pH sensor using a conventional 0.6- μ m CMOS process," *IEEE Sensors Journal*, vol. 4, no. 6, pp. 706-712, 2004.
- [117] R. R. Singh, L. Lian, A. Guenther, and R. Genov, "A CMOS-microfluidic chemiluminescence contact imaging microsystem," *IEEE Journal of Solid-State Circuits (JSSC)*, vol. 47, no. 11, pp. 2822-2833, 2012.
- [118] J. Farrell, F. Xiao, and S. Kavusi, "Resolution and light sensitivity tradeoff with pixel size," *Electronic Imaging 2006*, pp. 60690N-60690N-60698, 2006.
- [119] L. Shepherd, and C. Toumazou, "Weak Inversion ISFETs for ultra-low power biochemical sensing and real-time analysis," *Sensors and Actuators B: Chemical*, vol. 107, no. 1, pp. 468-473, 2005.
- [120] B. Nemeth, M. S. Piechocinski, and D. R. S. Cumming, "High-resolution real-time ion-camera system using a CMOS-based chemical sensor array for proton imaging," *Sensors and Actuators B: Chemical*, vol. 171, pp. 747-752, 2012.
- [121] C. Wai Pan, B. Premanode, and C. Toumazou, "An integrated ISFETs instrumentation system in standard CMOS technology," *IEEE Journal of Solid-State Circuits (JSSC)*, vol. 45, no. 9, pp. 1923-1934, 2010.
- [122] A. Manickam, R. Singh, N. Wood, L. Bingling, A. Ellington, and A. Hassibi, "A fully-electronic charge-based DNA sequencing CMOS biochip," *Symposium on VLSI Circuits (VLSIC)*, pp. 126-127, 2012.
- [123] H. M. Shapiro, "Using Flow Cytometers: Applications, Extensions, and Alternatives," *Practical Flow Cytometry*, pp. 443-542: John Wiley & Sons, Inc., 2005.
- [124] A. L. Givan, "The Sorting of Cells," *Flow Cytometry: First Principles*, pp. 159-174: John Wiley & Sons, Inc., 2001.
- [125] M. Brown, and C. Wittwer, "Flow cytometry: principles and clinical applications in hematology," *Clinical Chemistry*, vol. 46, no. 8, pp. 1221-1229, 2000.
- [126] G. P. Gupta, and J. Massagué, "Cancer metastasis: building a framework," *Cell*, vol. 127, no. 4, pp. 679-695, 2006.
- [127] M. A. Thompson, J. A. Aberg, J. F. Hoy, A. Telenti, C. Benson, P. Cahn *et al.*, "Antiretroviral treatment of adult HIV infection," *Jama*, vol. 308, no. 4, pp. 387-402, 2012.
- [128] N. Baumgarth, and M. Roederer, "A practical approach to multicolor flow cytometry for immunophenotyping," *Journal of immunological methods*, vol. 243, no. 1, pp. 77-97, 2000.
- [129] F. B. Myers, and L. P. Lee, "Innovations in optical microfluidic technologies for point-of-care diagnostics," *Lab on a Chip*, vol. 8, no. 12, pp. 2015-2031, 2008.
-

-
- [130] H. Zhu, S. O. Isikman, O. Mudanyali, A. Greenbaum, and A. Ozcan, "Optical imaging techniques for point-of-care diagnostics," *Lab on a Chip*, vol. 13, no. 1, pp. 51-67, 2013.
 - [131] D. Wlodkowic, and Z. Darzynkiewicz, "Rise of the micromachines: microfluidics and the future of cytometry," *Methods in cell biology*, vol. 102, pp. 105, 2011.
 - [132] X. Mao, A. A. Nawaz, S.-C. S. Lin, M. I. Lapsley, Y. Zhao, J. P. McCoy *et al.*, "An integrated, multiparametric flow cytometry chip using "microfluidic drifting" based three-dimensional hydrodynamic focusing," *Biomicrofluidics*, vol. 6, no. 2, pp. 024113, 2012.
 - [133] D. Huh, W. Gu, Y. Kamotani, J. B. Grotberg, and S. Takayama, "Microfluidics for flow cytometric analysis of cells and particles," *Physiological measurement*, vol. 26, no. 3, pp. R73, 2005.
 - [134] C. Yang, D. Erickson, and D. Psaltis, "OPTOFLUIDICS: Optofluidics enhances cytometry," *BioOptics World*, 2009.
 - [135] E. Schonbrun, S. S. Gorthi, and D. Schaak, "Microfabricated multiple field of view imaging flow cytometry," *Lab on a Chip*, vol. 12, no. 2, pp. 268-273, 2012.
 - [136] S.-C. Lin, P.-W. Yen, C.-C. Peng, and Y.-C. Tung, "Single channel layer, single sheath-flow inlet microfluidic flow cytometer with three-dimensional hydrodynamic focusing," *Lab on a Chip*, vol. 12, no. 17, pp. 3135-3141, 2012.
 - [137] J. C. McDonald, and G. M. Whitesides, "Poly (dimethylsiloxane) as a material for fabricating microfluidic devices," *Accounts of Chemical Research*, vol. 35, no. 7, pp. 491-499, 2002.
 - [138] Q. Wei, E. McLeod, H. Qi, Z. Wan, R. Sun, and A. Ozcan, "On-chip cytometry using plasmonic nanoparticle enhanced lensfree holography," *Scientific reports*, vol. 3, 2013.
 - [139] P. B. Catrysse, and B. A. Wandell, "Roadmap for CMOS image sensors: Moore meets Planck and Sommerfeld," *SPIE Electronic Imaging*, pp. 1-13, 2005.
 - [140] H. Rhodes, G. Agranov, C. Hong, U. Boettiger, R. Mauritzson, J. Ladd *et al.*, "CMOS imager technology shrinks and image performance," *IEEE Workshop on Microelectronics and Electron Devices*, pp. 7-18, 2004.
 - [141] C. C. Fesenmaier, Y. Huo, and P. B. Catrysse, "Optical confinement methods for continued scaling of CMOS image sensor pixels," *Optics express*, vol. 16, no. 25, pp. 20457-20470, 2008.
 - [142] G. Agranov, V. Berezin, and R. H. Tsai, "Crosstalk and microlens study in a color CMOS image sensor," *IEEE Transactions on Electron Devices*, vol. 50, no. 1, pp. 4-11, 2003.
 - [143] X. Huang, J. Guo, X. Wang, M. Yan, Y. Kang, and H. Yu, "A Contact-imaging based microfluidic cytometer with machine-learning for single-frame super-resolution processing," *PLOS ONE*, 2014.
 - [144] G.-B. Huang, Q.-Y. Zhu, and C.-K. Siew, "Extreme learning machine: theory and applications," *Neurocomputing*, vol. 70, no. 1, pp. 489-501, 2006.
-

-
- [145] W. T. Freeman, E. C. Pasztor, and O. T. Carmichael, "Learning low-level vision," *International journal of computer vision*, vol. 40, no. 1, pp. 25-47, 2000.
 - [146] L. An, and B. Bhanu, "Image super-resolution by extreme learning machine," *IEEE International Conference on Image Processing (ICIP)*, pp. 2209-2212, 2012.
 - [147] G.-B. Huang, H. Zhou, X. Ding, and R. Zhang, "Extreme learning machine for regression and multiclass classification," *IEEE Transactions on Systems, Man, and Cybernetics, Part B: Cybernetics*, vol. 42, no. 2, pp. 513-529, 2012.
 - [148] Z. Wang, A. C. Bovik, H. R. Sheikh, and E. P. Simoncelli, "Image quality assessment: from error visibility to structural similarity," *IEEE Transactions on Image Processing*, vol. 13, no. 4, pp. 600-612, 2004.
 - [149] O. Bowen, and C. S. Bouganis, "Real-time image super resolution using an FPGA," *International Conference on Field Programmable Logic and Applications (FPL)*, pp. 89-94, 2008.
 - [150] P. Sung Cheol, P. Min Kyu, and K. Moon Gi, "Super-resolution image reconstruction: a technical overview," *Signal Processing Magazine, IEEE*, vol. 20, no. 3, pp. 21-36, 2003.
 - [151] E. Choi, J. Choi, and M. G. Kang, "Super - resolution approach to overcome physical limitations of imaging sensors: An overview," *International Journal of Imaging Systems and Technology*, vol. 14, no. 2, pp. 36-46, 2004.
 - [152] M. Elad, and Y. Hel-Or, "A fast super-resolution reconstruction algorithm for pure translational motion and common space-invariant blur," *IEEE Transactions on Image Processing*, vol. 10, no. 8, pp. 1187-1193, 2001.
 - [153] W. Zhang, G. Ferguson, and S. Tatic-Lucic, "Elastomer-supported cold welding for room temperature wafer-level bonding," *IEEE International Conference on Micro Electro Mechanical Systems (MEMS)*, pp. 741-744, 2004.
 - [154] H. M. Wyss, D. L. Blair, J. F. Morris, H. A. Stone, and D. A. Weitz, "Mechanism for clogging of microchannels," *Physical Review E*, vol. 74, no. 6, pp. 061402, 2006.
 - [155] C. Bernabini, D. Holmes, and H. Morgan, "Micro-impedance cytometry for detection and analysis of micron-sized particles and bacteria," *Lab on a Chip*, vol. 11, no. 3, pp. 407-412, 2011.
 - [156] A. J. Lipton, H. Fujiyoshi, and R. S. Patil, "Moving target classification and tracking from real-time video," *IEEE Workshop on Applications of Computer Vision (WACV)*, pp. 8-14, 1998.
 - [157] M. Piccardi, "Background subtraction techniques: a review," *IEEE International Conference on Systems, Man and Cybernetics*, pp. 3099-3104, 2004.
 - [158] C. Cortes, and V. Vapnik, "Support-vector networks," *Machine learning*, vol. 20, no. 3, pp. 273-297, 1995.
 - [159] B. Yegnanarayana, "Artificial Neural Networks, PHI Learning Pvt," *Ltd., New Delhi, India*, 2004.
-

- [160] Y. Chen, A. A. Nawaz, Y. Zhao, P.-H. Huang, J. P. McCoy, S. J. Levine *et al.*, “Standing surface acoustic wave (SSAW)-based microfluidic cytometer,” *Lab on a Chip*, vol. 14, no. 5, pp. 916-923, 2014.
- [161] S. O. Isikman, W. Bishara, H. Zhu, and A. Ozcan, “Optofluidic tomography on a chip,” *Applied Physics Letters*, vol. 98, no. 16, pp. 161109, 2011.