

## **Copyright**

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the Permission of the Author.

**Biomedical Integrated Circuit Design**  
**for**  
**An Electro-Therapy Device**

*A Thesis Presented in Partial Fulfilment  
of The Requirements for The Degree of*

**Doctor of Philosophy**

*in*

*Electronics and Computer Engineering  
(Bioelectronics)*

*at*

*School of Engineering and Advanced Technology  
Massey University, Albany Campus, New Zealand*

*by*

**Ibtisam Abbas**

*Full name*

**(Ibtisam A. Abbas Al-Darkazly)**

***October 2017***

**In the name of God**  
**Most Gracious, Most Merciful**

Dedication

To my parents, whose boundless love and belief in me, from the core of my being, empowered me to be the woman that I am.

To my lovely children and to my talented kids, Zain, Taim and Yoseph, whom are the light in my life.

**“Our Actions Today are The Future of The Human Being.”**

## Abstract

A biomedical integrated circuit design (IC) is utilized for the development of a novel non-invasive electro-therapy device, for low frequency multi-channel biomedical stimulation to transform immune activity and induce anti-viral state. Biomedical integrated circuit design is an important branch of modern electronic engineering that uses the application of electronic engineering principles for biomedical disciplines, to develop bioelectronics devices that are implanted within the body and for non-invasive devices to improve patient's lives. These devices use the application of an electric field to stimulate reactions to restore normal cell functions and activate the cells to treat a variety of disorders or disease conditions. Bioelectronics devices can be designed for use as alternative treatments to overcome the deficiencies of several conventional medical treatments. It could potentially assist as drug-free relief when therapeutic drugs become ineffective, costly, with serious side effects and cannot be replaced, loss of future treatment options, and hence, life threatening, as for drug resistant Human immunodeficiency virus (HIV-1) patients.

Since the underlying mechanisms of the biological system and disease state is dominated by electrostatic interactions, specifically, the interaction between HIV-1 and the host cell that is predominantly by electrostatic interactions (protein charge-charge interaction) has an important role in its life cycle replication. At given pulses, the charge distribution and polarization of the electro-active protein molecules takes place, inducing conformation change which can enhance immune activity and inhibit the interaction of HIV-1 and host cells, disturbing its life cycle, leading to the mechanisms of the inactivation signal-induced virus death. These electrically induced protein transformations is used in this research as blood-cell treatment and as anti-HIV-1 electrotherapy.

Advances in bioelectronics technology, which involve new CMOS IC design, and in bio-electrochemistry science, which include cellular function, electro-active biomolecules and their responses, have contributed to this project to develop the concept of a novel electro-therapy device, for biomedical treatment applications. This involves understanding of the underlying mechanisms of the biological system and disease condition from an electronic engineer's point of view as well as the interface

between the electronic signal and the biological cells, and how electronic devices and circuitry directly communicate with the electro-active body tissue and blood cells.

This research project addresses the design and development of a novel energy-efficient miniature biomedical device using a new CMOS technology. It can generate, deliver and control an appropriate periodical low frequency electrical pulses, through the low-resistance skin surface to a patient's blood. The notable feature of such a smart device is its cellular specificity: the parameters of the generated electrical pulse which are designed and selected in order to stimulate only one particular type of tissue (blood) leaving the others unaffected. The device comprises a mixed-signal low power dual-band waveform generator (WFG) chip along with a novel two band tuning system. It was fabricated using Global Foundries (GF) 8RF-DM 130-nm CMOS process with a supply voltage of  $\pm 1V$  for the analog circuit and  $+1V$  for logic circuits. The WFG core (band I) can be tuned in the range 6.44 kHz - 1003 kHz through bias current adjustment, while a lower frequency (band II) in the range 0.1 Hz to 502 kHz can be provided digitally. Two WFG approaches, that comprise relaxation oscillators with different relaxation timing networks, have been developed for comparison.

Since the aim of this work is to transfer electrical signal in a specifically controlled fashion through the tissue, a novel low power active electrode-pair signal delivery system, compatible with human skin with high signal integrity, is developed. The circuit was fabricated in a 130-nm CMOS process using a low supply-voltage of  $+1.2V$  to deliver bi-phase square waveform signals from 16 selectable low-frequency channels. The individual active electrode can also be used to deliver mono-phase square/triangular waveform output signals. Accuracy, safety, low power, light-weight, miniature and low-cost characteristics are the main concerns. Being a miniature bioelectronics component with low power consumption, the proposed device is suitable both as a non-invasive and as an implantable biomedical device, in which WFG and electrodes circuitry can communicate with the electro-active biomolecule, strongly stimulating certain events in a complex biological system.

A theoretical analysis, experiment design and performance are carried out in *in-vitro* environments to examine the effect of the designed signal on human blood cellular proteins. Proteins that display a heterogeneous structure have various conductivities and permittivity (determining the interaction with the electrical field) and possess dielectric properties with a large conformation change, undergoing structural rearrangements in

response to cellular signals. The frequency-dependent dielectric present in proteins involves the redistribution and alignment of the proteins charged molecule and its polar molecule in response to an applied external electrical field can also induce conformation change. Interference polarization within proteins could interrupt the interaction between both sides of predominantly host cell proteins and of the HIV-1 infective envelope and its protein particles. This could disturb the signalling proteins for cell activation, and, hence, the virus cannot conjugate with the target cells and control the host cell protein activity. Since the virus is unable to reproduce out of a host cell, hence the virus cannot mutate and develop resistance easily, and use alternative binding and entry mechanisms as in the pharmacological approaches. After carefully studying the interaction of the HIV-1 virus and the host cell, with respect to signal transfer, CD4 receptor, co-receptors CCR5 and nuclear transport factor nucleoporins FG Nup153 proteins of the lymphatic system, which are essential targets for HIV-1 infection and its life cycle replication represent an attractive target to investigate in this research project. The activities of the underlying mechanism of the target cell are then examined utilizing immunofluorescence microscopy technique with specific fluorescent labelled antibodies, and accurate results are obtained with relatively low cost. The results demonstrated that the low frequency electrical pulse could inhibit virus attachment and fusion. It is also could provide a permeability barrier, that prevents the import and export of large macromolecule virus particles through the nuclear pore complex. These effects could induce an antiviral state for a period of time, and stop HIV-1 virus replication, with no potential risks and harm to the host cells, compared to the common drugs. This is promising for the conception of HIV-1 treatment in vivo. Although further investigations are required in order to fully use the application of electrical stimulation in vivo for treatment, the result is provides the necessary impetus for the applications of low frequency electrical stimulation on human immune response. This might offer important antiviral therapy against the most devastating pathogens in human history.

This doctoral research is not only of academic interest but also highly relevant to medical applications. It is considered potentially beneficial in the development of knowledge in advanced technology for electro-medical treatment devices, their design, structure and applications to extend life, and for future growth in the biotechnology industry, therefore beneficial for the patients, physicians and for humanity.

## **Acknowledgments**

The compilation of this research project would not have been possible without the support of others which is sincerely appreciated and thankfully acknowledged. Foremost, I would like to acknowledge and thank my supervisor Dr. Rezaul Hasan, for his valuable guidance, kindly encouragement and scholarly advice at various stages throughout my study. I would like to thank my supervisor Dr. Rezaul Hasan once again for facilitating the funding of this work through Massey University.

I would like to acknowledge the Massey University for financial support for the funding of this work. I also would like to acknowledge the Massey University Human Ethics Committee, Dr. Brian Finch, Human Ethics Chairs' Committee and Director, Mr. Jeremy Hubbard, Human Ethics Chairs' Committee: Southern A and to Ms. Patsy Broad, team leader. I would like to mention the facilities in the laboratory of Human Nutrition, School of Food and Nutrition (SoFN) laboratories, College of Health at Albany campus, Massey University for in-vitro biological tests as part of this work.

I am grateful to Dr. Pamela Von Hurst co-director of vitamin D research center for her keen interest in supporting the researcher and her prompt kind encouragement regarding lab facilities and safety. I would further like to thank Dr. Cath Conlon for providing me with a place in the Human Nutrition lab. I thank Mr. James Connell compliance support officer and Mr. PC Tong and Mrs. Rachel Liu for the use of lab facilities and for the health and safety induction in lab building 27 and lab building 10. I also thank profusely Dr. Peter Flanagan, National Medical Director, New Zealand Blood Service for providing a Blood sample. I also would like to acknowledge chip fabrication support from MOSIS.

I would like to thank the staff at School of Engineering and Advanced Technology for their support during my study. I thank Mr. Joe Wang for electronic lab facilities and Mr. Roukin Dmitri for the use of Linux programme. I also would like to thank my friends and colleagues for all of the valuable words of encouragement. I would further like to thank my thesis proof reader, Mrs. Diana Hibbert.

Finally, I would like to thank my family. Their love, sacrifice, patience and encouragement cannot be replaced by anything else in this world. I'm forever grateful to them.

# Table of Contents

|   |           |
|---|-----------|
| <b>Abstract.....</b>  | iii       |
| <b>Acknowledgements.....</b>  | vi        |
| <b>Table of Contents.....</b>   | vii       |
| <b>List of Abbreviations.....</b>   | xii       |
| <b>List of Symbols.....</b>   | xiv       |
| <b>List of Figures.....</b>   | xv        |
| <b>List of Tables.....</b>  | xxiii     |
| <b>Chapter 1. Introduction .....</b>  | 1         |
| 1.1 Biomedical Integrated Circuit Design .....                                | 1         |
| 1.2 Relevance.....  | 6         |
| 1.2.1 Virus Replication.....  | 7         |
| 1.3 Develop Concept .....   | 10        |
| 1.4 Research Goals.....   | 16        |
| 1.5 Scope of This Study.....  | 16        |
| 1.6 Thesis Overview.....  | 20        |
| <b>Chapter 2. Overview of The System Model, Theory, Design and Approaches</b> | <b>23</b> |
| 2.1 Introduction.....   | 23        |
| 2.2 Waveform Generator Overview.....  | 24        |
| 2.3 Basic Theory of Oscillator.....   | 26        |
| 2.3.1 Oscillators Approaches.....   | 28        |
| 2.3.2 Relaxation Oscillator Architecture.....                                 | 30        |
| 2.4 Hysteresis Schmitt Trigger Concept.....                                   | 30        |
| 2.5 Principles of Operation of Typically WFG Circuit.....                     | 33        |
| 2.5.1 Theory of WFG Based on RC Network.....                                  | 33        |
| 2.5.2 Theory of WFG Based on Integrator.....                                  | 35        |
| 2.6 Waveform Generator Approaches.....  | 36        |
| 2.6.1 Relaxation Timing Network Approaches.....                               | 46        |
| 2.6.1.1 Passive RC Approaches.....  | 46        |
| 2.6.1.2 Active Integrator Building Block Approaches.....                      | 48        |



|  |  |     |
|--|--|-----|
| 2.7  | Tuning Circuit Approaches.....                                     | 53  |
| 2.8  | Digital Model for Frequency Divider.....                           | 55  |
| 2.8.1  | Frequency Divider Theory.....                                      | 56  |
| 2.8.2  | Flip Flop Approaches.....  | 57  |
| 2.8.3  | Sources of Power Dissipation In A Digital Model.....               | 64  |
| 2.9  | Active Electrode.....  | 67  |
| 2.10   | Conclusion.....  | 70  |
| <br>   |  |     |
| <b>Chapter 3. Design Criteria, Implementation and Fabrication of Waveform</b>  |  |     |
| <b>Generator Circuit for Extra Low-Frequency CMOS Micro-</b>                   |  |     |
| <b>Power Applications .....</b>  |  |     |
|  |  | 72  |
| 3.1  | Introduction.....  | 72  |
| 3.2  | Trade-off of Low Power CMOS WFG Design Analysis.....               | 73  |
| 3.3  | Design Criteria of CMOS ELF WFG Circuit.....                       | 79  |
| 3.4  | Circuit Design and Topology of The ELF WFG.....                    | 81  |
| 3.5  | Circuit Operation of The ELF WFG.....                              | 82  |
| 3.6  | Simulation and Performance Analyses of The ELF WFG.....            | 88  |
| 3.6.1  | Amplitude Control of The ELF WFG.....                              | 91  |
| 3.6.2  | Frequency Control of The ELF WFG.....                              | 94  |
| 3.7  | Layout and Fabrication of The ELF WFG.....                         | 96  |
| 3.8  | Experimental Results of The ELE WFG.....                           | 102 |
| 3.9  | Conclusion.....  | 104 |
| <br>   |  |     |
| <b>Chapter 4. Design Criteria, Implementation and Fabrication of Dual-Band</b> |  |     |
| <b>CMOS Waveform Generator With Ultra-Wide Low-Frequency</b>                   |  |     |
| <b>Tuning Range.....</b>   |  |     |
|  |  | 106 |
| 4.1  | Introduction.....  | 106 |
| 4.2  | Design Criteria of CMOS Dual-Band WFG <sub>INT</sub> .....         | 108 |
| 4.3  | Circuit Design and Topology of The WFG <sub>INT</sub> .....        | 111 |
| 4.3.1  | WFG <sub>INT</sub> Circuit Design.....                             | 111 |
| 4.3.2  | Hysteresis Schmitt Trigger Circuit of The WFG <sub>INT</sub> ..... | 112 |
| 4.3.3  | Gm-C Integrator Realization.....                                   | 113 |

|         |  |     |
|---------|--|-----|
| 4.3.4   | WFG <sub>INT</sub> Circuit Operation.....  | 117 |
| 4.3.5   | Components Sizing for Low Power & Low Frequency<br>WFG <sub>INT</sub> Design.....      | 121 |
| 4.4     | Frequency Tuning Technique.....  | 125 |
| 4.4.1   | Analog Tuning Model.....   | 125 |
| 4.4.2   | Digital Model Implementation.....  | 126 |
| 4.4.2.1 | Clock and Clock_Bar.....   | 126 |
| 4.4.2.2 | The Frequency Division Circuit.....  | 126 |
| 4.4.2.3 | Multiplexors And Path Selector.....  | 128 |
| 4.5     | Simulation and Performance Analyses Of WFG <sub>INT</sub> .....                        | 132 |
| 4.5.1   | Robustness of The WFG <sub>INT</sub> Circuit.....                                      | 135 |
| 4.5.1.1 | Temperature Variation.....   | 135 |
| 4.5.1.2 | Eye Diagram Analysis.....  | 135 |
| 4.6     | Simulation Results of The Digital Model.....   | 137 |
| 4.6.1   | Clock And Clock_Bar.....   | 139 |
| 4.6.2   | FD circuit.....  | 140 |
| 4.7     | Layout and Fabrication of The WFG <sub>INT</sub> .....                                 | 146 |
| 4.8     | Experimental Results of The WFG <sub>INT</sub> .....                                   | 150 |
| 4.8.1   | Amplitude Control of The WFG <sub>ING</sub> .....                                      | 154 |
| 4.8.2   | Frequency Control of The WFG <sub>ING</sub> .....                                      | 155 |
| 4.9     | Optimization Flowchart of The WFG <sub>ING</sub> .....                                 | 159 |
| 4.10    | Comparison of The WFG <sub>INT</sub> With ELF WFG and With<br>Other Published WFG..... | 161 |
| 4.11    | Conclusion.....  | 163 |

|   |  |     |
|---|--|-----|
| <b>Chapter 5. Design Criteria, Implementation and Fabrication of a Low-<br/>Power CMOS Active-Electrode-Pair For Low-Frequency Multi-<br/>Channel Biomedical Stimulation.....</b> |  | 165 |
| 5.1   | Introduction.....  | 165 |
| 5.2   | Design Criteria for Active Electrode.....                | 169 |
| 5.3   | Circuit Design and Topology of The Active Electrode..... | 170 |

|  |            |
|--|------------|
| 5.3.1 Active Electrode Circuit Analysis.....   | 171        |
| 5.4 Simulation And Performance Analyses of The Active Electrode.....   | 174        |
| 5.5 Layout and Fabrication of The Active Electrode.....  | 178        |
| 5.5.1 Experiment Results of The Active Electrode.....  | 180        |
| 5.6 Conclusion.....  | 185        |
| <b>Chapter 6. In Vitro Biological Experiment Design And Performance To Investigate The Effect of Low Frequencies Electrical Pulses on The Human Blood Cells.....</b> | <b>186</b> |
| 6.1 Introduction.....  | 186        |
| 6.2 Chemokine Receptor CCR5.....   | 189        |
| 6.2.1 CCR5 Protein Structure.....  | 190        |
| 6.3 The Nuclear pore complex (NPC).....  | 193        |
| 6.4 Frequency–Dependent Polarization.....  | 202        |
| 6.5 In Vitro Biological Tests.....   | 205        |
| 6.5.1 Materials and Procedure.....   | 205        |
| 6.5.2 Electrical Simulation Procedure.....   | 207        |
| 6.5.3 Immunofluorescence Microscopy Assay.....   | 208        |
| 6.5.3.1 Immunofluorescence Cell Staining Procedure.....  | 209        |
| 6.6 Electrical Stimulation Results.....  | 214        |
| 6.6.1 Expression of CD4 and CCR5.....  | 214        |
| 6.6.2 CCR5 N-Terminal Conformation Epitopes.....   | 216        |
| 6.6.3 Distribution of FGNup153.....  | 218        |
| 6.7 Discussions on Experimental Results.....   | 223        |
| 6.8 Conclusion.....  | 229        |
| <b>Chapter 7. Conclusion.....</b>  | <b>231</b> |
| 7.1 Summary.....   | 231        |
| 7.2 Contributions of This Doctoral Research.....   | 243        |
| 7.3 Recommendations for Future Research.....   | 246        |
| <b>References.....</b>   | <b>249</b> |

|                    |                                |     |
|--------------------|--------------------------------|-----|
| <b>Appendix A.</b> | <b>Supplementary Documents</b> | 282 |
|                    | <b>Ethic Approval</b>          | 283 |
|                    | <b>List of Publications</b>    | 284 |

## List of Abbreviations

|         |  |
|---------|--|
| AA      | Amino Acid                             |
| Ab      | Antibody                               |
| D       | Aspartic Acid                          |
| BJT     | Bipolar Technology                     |
| BSA     | Bovine Serum Albumin                   |
| CCII    | Current Conveyor                       |
| CFOA    | Current Feedback Operational Amplifier |
| Clk     | Clock                                  |
| Clk_bar | Clock_bar                              |
| CM      | Current-Mode                           |
| CSE     | Clocked Storage Element                |
| D-FF    | D-Flip Flop                            |
| DRC     | Design Rule Checking                   |
| ELF     | Extra Low Frequency                    |
| FBS     | Fetal Bovine Serum                     |
| FD      | Frequency Divider                      |
| FF      | Flip Flop                              |
| FG      | Phenylalanine-Glycine                  |
| h       | Hour                                   |
| HCl     | Hydrochloric acid                      |
| HIV-1   | Human immunodeficiency virus           |
| IC      | Integrated Circuit                     |
| Kap     | Karyopherin                            |
| LVS     | Layout Versus Schematic                |
| mAbs    | Monoclonal Antibodies                  |
| min     | Minute                                 |
| MLF     | Moderate Low Frequency                 |
| MUX     | Multiplexer                            |
| NES     | Nuclear Export Signal                  |
| NLS     | Nuclear Localization Signal            |
| nm      | Nano-metric                            |

|                     |   |
|---------------------|---|
| NPC                 | Nuclear pore complex                                  |
| Op-Amp              | Operational Amplifier                                 |
| OTA                 | Operational Trans-conductance Amplifiers              |
| PFA                 | Paraformaldehyde                                      |
| PG                  | Pass Gate   |
| PIC                 | Pre-Integration Complex                               |
| PS                  | Path Selector   |
| PVT                 | Process And Temperature Variation                     |
| Q                   | Glutamine   |
| SC                  | Stratum Corneum                                       |
| SDL                 | Schematic-Driven Layout                               |
| ST                  | Schmitt Trigger                                       |
| STG                 | Stage   |
| TG                  | Transmission-Gate                                     |
| TGFF                | Transmission-Gate Flip Flop                           |
| VTC                 | Voltage Transfer Characteristic                       |
| WFG                 | Waveform Generator                                    |
| WFG <sub>INTG</sub> | Waveform Generator Based on Integrator Timing Network |
| Y                   | Tyrosine  |

## List of Symbols

|            |                                |                            |
|------------|--------------------------------|----------------------------|
| $A$        | Area                           | Meter Square               |
| $C$        | Capacitor                      | Farad                      |
| $C_{ox}$   | Gate oxide capacitance         | Farad                      |
| $gm$       | Trans-conductance              | Microampere/microvolt      |
| $W$        | Channel width of the MOSFET    | Micro-meter                |
| $L$        | Channel length of the MOSFET   | Micro-meter                |
| $r_o$      | Output resistance of MOSFET    | Ohms                       |
| $\mu_n$    | Electron mobility              | Meter square/Volts seconds |
| $I_B$      | Bias Current of MOSFET         | Microamperes               |
| $I_D$      | DC Drain current of MOSFET     | Microamperes               |
| $V_C$      | Capacitor Voltage Output       | Volts                      |
| $V_{DD}$   | Positive Supply Voltage        | Volts                      |
| $V_{INT}$  | Integrator Voltage Output      | Volts                      |
| $V_O$      | Output Voltage                 | Volts                      |
| $V_{SS}$   | Negative Supply Voltage        | Volts                      |
| $V_{GS}$   | Gate-source voltage of MOSFET  | Volts                      |
| $V_{LTH-}$ | Lower Threshold Voltage        | Volts                      |
| $V_{ST}$   | Schmitt Trigger Voltage Output | Volts                      |
| $V_{TH}$   | Threshold voltage              | Volts                      |
| $V_{UTH+}$ | Upper Threshold Voltage        | Volts                      |
| $V_{in}$   | Input Voltage                  | Volts                      |
| $V_{sat-}$ | Low Negative Saturation Level  | Volts                      |
| $V_{sat+}$ | High Positive Saturation Level | Volts                      |
| $R$        | Resistor                       | Ohms                       |
| $P$        | Power                          | Watts                      |
| $f$        | Frequency                      | Hertz                      |
| $\tau$     | Time constant                  | Seconds                    |
| $T$        | Time Period                    | Seconds                    |
| $\omega$   | Angular frequency              | Radians per second         |

## List of Figures

|             |  |    |
|-------------|--|----|
| Figure 1.1: | Schematic diagram representing the development of a variety of implantable and non-invasive biomedical devices in the real world. These devices use the application of an electric field with an appropriate electrical signal and specific waveform and frequency to be applied internally or externally to a particular area of the body, to treat a variety of disorders or disease conditions, and to improve patients' lives..... | 5  |
| Figure 1.2: | The primary protein structure, (a) amino acid structure, and (b) amino acids are connected together by a covalent linkage called a peptide bond to form polypeptide polymer chains and to build up the primary structure of a protein.....   | 10 |
| Figure 1.3: | The development and design steps of electro-therapy concept and device of this research project, (a) block diagram, and (b) Schematic diagrams.....  | 15 |
| Figure 2.1: | Block diagram of a simple positive feedback system.....  | 27 |
| Figure 2.2: | Schematic diagram of a simple oscillatory system.....  | 27 |
| Figure 2.3: | Schmitt Trigger, (a) basic Schmitt trigger circuit and (b) voltage transfer characteristic of the Schmitt Trigger.....   | 31 |
| Figure 2.4: | Comparison between the output voltage transfer characteristic of the comparator and the hysteresis Schmitt Trigger circuit, (a) comparator response to noisy signal, and (b) hysteresis Schmitt Trigger response to noisy signal.....  | 32 |
| Figure 2.5: | A basic block diagram of a typical WFG circuit, based on a RC relaxation timing network.....   | 34 |
| Figure 2.6: | A basic block diagram of a typical WFG circuit based on a hysteretic Schmitt Trigger and an integrator relaxation timing network.....  | 35 |
| Figure 2.7: | Waveform generator architectures based on, (a) single differential N-MOSFET OTA, (b) two differential N-MOSFET OTAs.....   | 41 |
| Figure 2.8: | WFG architectures based on discrete LM3080 ICs OTAs and one  |    |



|              |   |    |
|--------------|---|----|
|              | comparator.....   | 42 |
| Figure 2.9:  | WFG architectures based on one OTA and a two-stage comparator.....  | 42 |
| Figure 2.10: | WFG architectures based on a dual output DO-OTA, using two single-ended commercial CA3080 OTA ICs.....  | 43 |
| Figure 2.11: | WFG architectures based on (a) three CMOS OTAs, (b) using three commercial available LM13600 ICs.....   | 44 |
| Figure 2.12: | Op-Amp-RC integrator (a) first order Op-Amp-RC Integrator, (b) digitally controlled switched-capacitor matrices for tuning the time constant of the Op-Amp-RC integrator circuit.....   | 49 |
| Figure 2.13: | Gm-C integrator (a) first order integrator, (b) a lossy integrator...   | 50 |
| Figure 2.14: | Resistive tuning techniques, voltage controls a bank of MOSFETs.....  | 53 |
| Figure 2.15: | Schematic diagram of the frequency divider for “divide-by-2” (/2) frequency division circuit with its waveform output.....  | 56 |
| Figure 2.16: | Schematic diagram of the logic gate (a) a simple PMOS and NMOS pass transistor and (b) a CMOS transmission gate and its logic symbol.....   | 63 |
| Figure 2.17: | Schematic diagram of dynamic switching power dissipation in CMOS inverter.....  | 64 |
| Figure 2.18: | Types of electrodes: (a) passive electrodes, and (b), active electrode, using commercial Op-Amps with Ag/AgCl transducer for biomedical applications.....   | 68 |
| Figure 3.1:  | Trans-conductance amplifier (a) a single common source MOSFET transistor operating in the saturation region as a current source, (b) the small-signal model.....  | 74 |
| Figure 3.2:  | CMOS Operational trans-conductance amplifier, (a) schematic diagram (b) the small signal model of the differential amplifier with a current mirror load.....  | 77 |
| Figure 3.3:  | Proposed, ELF WFG. Based on the periodical charging and discharging operation of the capacitor C, ELF WFG circuit, provides a periodical square waveform output signal at $V_o$ and an exponential waveform at $V_c$ with no input..... | 81 |

|              |  |     |
|--------------|--|-----|
| Figure 3.4:  | Waveforms in the proposed ELF WFG.....   | 83  |
| Figure 3.5:  | Simulated transient output waveforms for ELF WFG for the first design.....   | 90  |
| Figure 3.6:  | Amplitude (P–P) variation with the bias current $IB3$ and resistor $R1$ , (a) amplitude (P–P) variation with $IB3$ for the two ELF WFG designs, (b) amplitude (P–P) variation with $R1$ for the first design and (c) amplitude (P–P) variation with $R1$ for the second design.....                    | 93  |
| Figure 3.7:  | Frequency tuning with bias current $IB1$ for the two ELF WFG designs.....  | 94  |
| Figure 3.8:  | Frequency tuning with resistor $R2$ , (a) for the first ELF WFG design, (b) for the second ELF WFG design.....   | 95  |
| Figure 3.9:  | The layout of on-chip identical units OPRPP and OPRRP resistors, in series.....  | 97  |
| Figure 3.10: | Layout for MIM capacitor.....  | 98  |
| Figure 3.11: | The layout for the two WFG circuit (design_1 and design_2). The layout on top right corner and bottom right corner represent the complete layout for the two WFG circuit including the on-chip designed MIMCAP for MLF WFG with 40pF, and for ELE WFG with 1nF for design _1 for area comparison.....  | 100 |
| Figure 3.12: | A close view of the complete layout (excluding the capacitor) for the two ELF WFG circuit, for chip fabrication (a) for the first design, (b) for the second design. The top is the Schmitt Trigger circuit, while the rectangular box is the combination of different types of on-chip resistors..... | 101 |
| Figure 3.13: | Package of the two ELF WFG circuit in PGA.....   | 102 |
| Figure 3.14: | Chip photo-micrograph illustrates the location of the integrated two ELE WFG circuit.....  | 102 |
| Figure 3.15: | Chip outputs of the CMOS ELE WFG for the first design.....   | 103 |
| Figure 4.1:  | Block diagram of the proposed mixed-signal CMOS waveform generator. Oscillation frequency of WFG core circuit is $f_{WFG}$ (band I) and the digitally channelized selectable output frequency is $f_o$ (band II).....  | 107 |

|              |   |     |
|--------------|---|-----|
| Figure 4.2:  | A schematic diagram of the core square/triangular WFG <sub>INT</sub> based on gm-C integrator with clock and clock_bar generator.....   | 112 |
| Figure 4.3:  | A single stage gm-C integrator based on CMOS OTA, (a) circuit implementation, (b) integrator symbol and (c) building blocks diagram.....  | 113 |
| Figure 4.4:  | Schematic diagram of the WFG <sub>INT</sub> circuit operation, (a) transfer characteristic of the Schmitt trigger design, and (b) the square and triangular waveforms of the designed WFG <sub>INT</sub> circuit..... | 117 |
| Figure 4.5:  | The schematic diagram of the designed TGMS D-FF for a divide-by/2 frequency division circuit.....   | 128 |
| Figure 4.6:  | The implementation of the designed TGMS D-FF for a divide-by/2 frequency division circuit.....  | 128 |
| Figure 4.7:  | Multiplexers and path selector along with output driving circuit...   | 130 |
| Figure 4.8:  | The complete architecture of the dual-band 16-channel mixed-signal WFG <sub>ING</sub> with ultra-wide low frequency tuning range.....   | 131 |
| Figure 4.9:  | Simulated transient square waveform output of the WFG <sub>INT</sub>  | 134 |
| Figure 4.10: | Spectrum analysis of the square waveform signal ( $f_{WFG} = 17$ kHz) in simulation profile.....  | 134 |
| Figure 4.11: | Simulated transient triangular waveform at the integrator output..  | 135 |
| Figure 4.12: | WFG <sub>INT</sub> circuit analysis for the frequency stability with temperature variations.....  | 137 |
| Figure 4.13: | Eye diagram simulation results for the designed WFG <sub>INT</sub> .....  | 137 |
| Figure 4.14: | Simulated transient response of the clock and clock_bar generator.....  | 139 |
| Figure 4.15: | The 16 channel output square waveforms digitally selectable through MUX1, MUX2 and PS.....  | 141 |
| Figure 4.16: | Power versus generalized width $W_N$ .....  | 143 |
| Figure 4.17: | Power dissipation versus supply voltage of the FD circuit.....  | 144 |
| Figure 4.18: | WFG <sub>ING</sub> frequency versus supply voltage.....   | 144 |
| Figure 4.19: | The simulated speed-power trade-off of the FD circuit.....  | 145 |
| Figure 4.20: | The simulation result for power and frequency versus V_tune.....  | 145 |
| Figure 4.21: | The complete layout (including the capacitor) for the (mixed-signal) dual-band WFG <sub>INT</sub> circuit, (a) the overall layout of the  |     |

|              |   |     |
|--------------|---|-----|
|              | mixed-signal WFG <sub>INT</sub> circuit, (b) show close views of the WFG <sub>INT</sub> core circuit, (c) D-FF, (d) MUX1, and (e) PS circuit.....   | 149 |
| Figure 4.22: | Package of the analog WFG <sub>INT</sub> and the digital model circuit in PGA.....  | 150 |
| Figure 4.23: | Chip photo-micrograph illustrates the location of the integrated mixed-signal dual-band WFG <sub>INT</sub> circuit.....   | 150 |
| Figure 4.24: | Experimental measurement set up for the fabricated oscillator.....  | 151 |
| Figure 4.25: | Measured chip outputs, (a) square waveform of the core WFG <sub>INT</sub> , (b) FFT spectrum analysis of the generated square waveform signal, (c) triangular waveform of the integrator, and (d) clock and clock_bar translation outputs.....  | 153 |
| Figure 4.26: | Amplitude (P–P) variation comparing measured results and simulation, (a) with V_tune bias current <i>IB3</i> , and, (b) with <i>R</i> .   | 155 |
| Figure 4.27: | The measured profile values of the oscillation frequency vs. V_tune of the WFG <sub>INT</sub> output signal, compared with the simulation profile values and the related results for 10 pF, 100 pF and 1000 pF load capacitor values respectively.....  | 157 |
| Figure 4.28: | Optimization flowchart of the designed CMOS dual-band 16-chanal mixed-signal waveform generator.....  | 160 |
| Figure 5.1:  | Human skin layer.....   | 166 |
| Figure 5.2:  | Current response of skin to the application of a square-wave voltage pulse.....   | 167 |
| Figure 5.3:  | Proposed adaptive biased CMOS active electrode circuit, (a) circuit implementation, with input stage bias current set dynamically by two mechanisms, first, by the simple current mirror and secondly, by applying adaptive biasing, and (b) equivalent circuit representing the voltage follower active electrode circuit..... | 170 |
| Figure 5.4:  | Simulated transient response of individual active electrode buffer to an input square-wave pulse of a 100ms duration and +1V amplitude (the <i>V<sub>OPS</sub></i> output of the PS in chapter 4) for <i>RL</i> = 50kΩ.   | 175 |
| Figure 5.5:  | The configuration for bio-potentials signals application,   |     |

|              |  |     |
|--------------|--|-----|
|              | employing two identical active electrodes.....   | 177 |
| Figure 5.6:  | Simulated transient response of the dual active electrode circuit driving a 50k $\Omega$ floating load in a differential fashion displaying a transmitted bi-phase square waveform with, (a) approximately $\pm$ 1V differential output voltage, and (b) $\pm$ 40 $\mu$ A differential output current.....   | 178 |
| Figure 5.7:  | The two identical active electrode circuits (a) layout, (b) package of the microchip and (c) Chip photo-micrograph of the fabricated die.....  | 180 |
| Figure 5.8:  | Experimental set-up for the active electrode chip output waveform measurements.....  | 181 |
| Figure 5.9:  | Oscilloscope trace of the differential output waveform signal of the dual identical active electrode circuits.....   | 181 |
| Figure 5.10: | A proposed proto-type electro-bio-stimulation system using the fabricated active electrode-pair complete with (a) mixed-signal CMOS waveform generator, WFG <sub>ING</sub> (IC1 in chapter 4), (b) the active electrode-pair (IC2), and, (c) solar panel charged regulated power-supply. The complete encapsulated bio-medical device with electrode contacts is shown in (d)..... | 184 |
| Figure 6.1:  | Protein structure of co-receptor CCR5 and its sequence. The image outlines the residues of N-terminus, and C-terminus, residues of the 7-TM region and of the extracellular (EL) and intracellular loop (IL) regions respectively.....   | 190 |
| Figure 6.2:  | Nuclear pore complex structure.....  | 195 |
| Figure 6.3:  | A heterogeneous tripartite structure of FG <sub>Nup153</sub> , comprises three different domains, N-terminal domain, zinc finger domain, and C-terminal domain.....  | 197 |
| Figure 6.4:  | Nuclear pore complex model for protein import and export of large macromolecular proteins that are recognized by nuclear transport receptors (NTRs) and passage them through the central channel.....  | 201 |
| Figure 6.5:  | The electrical test glass chamber with stainless steel wire electrodes.....  | 206 |

|              |   |     |
|--------------|---|-----|
| Figure 6.6:  | Experimental set up for electrical stimulation test. Healthy human buffy coat samples were exposed for 2h to low frequency bipolar square waveform pulses of 5Hz, 10Hz and 1000kHz with 1Vpp in a 30ml capacity glass chamber, 3cm in diameter, with stainless steel wire electrodes.....   | 207 |
| Figure 6.7:  | Purifying and coating coverslips with HCl and poly-lysine respectively, to assure cells adhesion.....   | 208 |
| Figure 6.8:  | Cells adhesion on coverslips. Buffy coat samples spread and plated on coated coverslips that are mounted on Parafilm to prevent the coverslips from moving, and then incubated in oven for 30 min at 37 °C.....   | 209 |
| Figure 6.9:  | The steps of experimental procedure for immunofluorescence cells staining.....  | 212 |
| Figure 6.10: | Experimental setup for acquiring images. (a) A Carl Zeiss Axio Star plus microscope with AxiosVison software, and (b) the tested and most successful sealed coverslips used in this experiment.....   | 213 |
| Figure 6.11: | Cell surface expression of CD4 (green) and CCR5 (yellow) in human healthy buffy coat samples, for unstimulated (A) cells and for electrically stimulated cells (B) in response to low frequency electrical simulation conditions of 5Hz and 10Hz with 1V for 2h, analysed by immunofluorescence microscope. The intensity and distribution of the fluorescence on the cell membrane and cell surface represents the concentration of CD4 expression and CCR5 expression for unstimulated cells as shown in (b) and (f), and for electrically stimulated cells of 5Hz and 10Hz as shown in (c), (d), (g) and (h) respectively. (a) and (b) images display a wider view of the CD4 and CCR5 cell populations..... | 215 |
| Figure 6.12: | Immunofluorescence assay for cellular distribution and binding activity of the healthy human CCR5 receptor. Leukocyte-rich buffy coat cells were assayed with mAb 3A9 followed by Alexa Fluora 555 labelled goat anti-mouse IgG directed against the N-terminal domain of CCR5 to study the binding activities of 3A9   |     |

epitope in this region, in response to a low frequency electrical field. The images (a), (b) and (c) show a wider view of the cell population, the binding activity of unstimulated cells (A), CCR5 (yellow) in (d) with 3A9 (red) in (e) is shown in orange (merge) in (f), while (g) represents IgG as a control, (h) and (i) represents the CCR5 (yellow) in response to electrical stimulation (B) of 5Hz and 10Hz respectively..... 217

Figure 6.13: The immunofluorescence microscope images of the distribution and pattern of the FGNup153. The images DAPI (blue) in (a) and (d) with Nup153 (red) in (b) and (e) is shown in purple (merge) in (c) and (f), displaying a wider view of the cell population for unstimulated cells (A) respectively. The co-localization of FGNup153 with mAb for (B) cells electrically stimulated with 5Hz and 10Hz incubated for 15min at -20°C in cold 100% methanol are displayed in (g), (h), (i) and (j), (k), and (l), respectively. For cells stimulated with 5Hz and 10Hz (green) that were fixed in 4% PFA and permeabilized by incubating for 5 min at room temperature with 0.5% Triton X-100 the data is not shown..... 222

## Lists of Tables

|            |  |     |
|------------|--|-----|
| Table 2.1: | A comparison between capacitor values and corresponding kT/C noise.....  | 47  |
| Table 3.1: | Transistor dimensions for the two ELF WFG designs (1 and 2)...   | 89  |
| Table 3.2: | Component values for the two ELF WFG designs (1 and 2).....  | 89  |
| Table 3.3: | Comparison of the simulation performance of the first ELF WFG design (design 1) with the second ELF WFG design (design 2)...   | 91  |
| Table 4.1: | The power and area versus generalized transistor width for the designed FD circuit.....  | 143 |
| Table 4.2: | Simulation results of the outputs of the dual band WFG <sub>INT</sub> designed circuit.....  | 146 |
| Table 4.3: | The tuning range of the oscillator frequency for the WFG <sub>INT</sub> core (band I) for C=10pF, with V-tune varied from -0.9V to +0.9V, and the equivalent oscillator frequency the circuit can provide, with 16 channel FD circuit (band II)..... | 158 |
| Table 4.4: | Comparison of the proposed WFG <sub>INT</sub> with ELF WFG of this work and with some other solutions in the reported literature.....  | 162 |
| Table 5.1: | Performance and comparison of the proposed active electrode with the previous reported   | 183 |