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A Multiplexed Impedance Analyser for Biosensing Applications

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Report submitted in partial fulfilment of the requirements of the module Project (E) 448 for the degree Baccalaureus in Engineering in the Department of Electrical and Electronic Engineering at Stellenbosch University.

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October 2025

Acknowledgements

I would like to thank my dog, Muffin. I also would like to thank the inventor of the incubator; without him/her, I would not be here. Finally, I would like to thank Dr Herman Kamper for this amazing report template.

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Abstract

English

The English abstract.

Afrikaans

Die Afrikaanse uittreksel.

Contents

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Nomenclature

Variables and functions

$p(x)$	Probability density function with respect to variable x.
$P(A)$	Probability of event A occurring.
ε	The Bayes error.
ε_u	The Bhattacharyya bound.
B	The Bhattacharyya distance.
s	An HMM state. A subscript is used to refer to a particular state, e.g. s_i refers to the i^{th} state of an HMM.
$\mathbf{S}$	A set of HMM states.
$\mathbf{F}$	A set of frames.
$\mathbf{o}_f$	Observation (feature) vector associated with frame f.
$\gamma_s(\mathbf{o}_f)$	A posteriori probability of the observation vector $\mathbf{o}_f$ being generated by HMM state s.
μ	Statistical mean vector.
Σ	Statistical covariance matrix.
$L(\mathbf{S})$	Log likelihood of the set of HMM states $\mathbf{S}$ generating the training set observation vectors assigned to the states in that set.
$\mathcal{N}(\mathbf{x}\|\mu, \Sigma)$	Multivariate Gaussian PDF with mean μ and covariance matrix Σ.
a_{ij}	The probability of a transition from HMM state s_i to state s_j.
N	Total number of frames or number of tokens, depending on the context.
D	Number of deletion errors.
I	Number of insertion errors.
S	Number of substitution errors.

Acronyms and abbreviations

ADC analogue-to-digital converter

DAC digital-to-analogue converter

DUT device-under-test

EIS electrochemical impedance spectroscopy

LPF low-pass filter

MCU microcontroller unit

PGA programmable gain amplifier

POC point-of-care

TIA transimpedance amplifier

Chapter 1

Introduction

1.1. Background

Biosensors are defined as devices that measures biological and chemical reactions through the use of a physical transducer mechanism which in turn generates signals porportional to the concentration of an analyte in a sample. [2] This allows for the detection of various biological elements such as biomarkers which can be used to monitor health conditions or diagnose diseases.

Designing a low-cost easy to use device to take readings from biosensors, would thus provide a valuable tool for the early screening of diseases such as cancer. Changing wording and goals to be focused on point of care screening rather than analysis/detection

This would decrease the need for expensive labratory testing and allow for lower cost and more regular testing of patients using a point-of-care device. Having a multiplexed device, able to take readings from multiple biosensors, would allow a single blood sample to be taken and multiple tests to be run on it with minimum involvement from a healthcare professional.

1.2. Project Objectives

This is a multifaceted project that aims to develop a low-cost, easy-to-use device for reading biosensors. The system design objectives for the device include:

- Developing a circuit that allows a microcontroller to read a biosensor through the use of Electrochemical Impedance Spectroscopy (EIS). This entails conditioning the sinusoidal signal generated by a DAC and measuring the current response from the biosensor through an ADC.
- This measurement circuitry needs to be multiplexed to allow for multiple biosensors to be read in turn without the need for human intervention.
- The microcontroller should be able to filter and process the biosensor data to determine the concentration of the analyte in the sample.

- The device needs to be able to communicate these results in a clear and user-friendly manner. This will be done through the use of a LCD screen on the device as well as a web interface that can be accessed through a smartphone or computer.
- The device should be battery powered, low-cost and easy to use, thus allowing for widespread adoption and use in various environments.

1.3. Project Scope

NOT THE DESIGN OF Biosensors NOT TESTING WITH REAL BLOOD (We arent qualified for that)

1.4. Chapter Overview

Chapter 1: Introduction

Provides an overview of the motivation, background, objectives, scope, and structure of the report.

Chapter 2: Literature Review

Reviews relevant research and technologies in speech recognition and neural networks.

Chapter 3: Methodology

Describes the methods and approaches used in the project, including data collection and model development.

Chapter 4: Experiments and Results

Presents experimental setup, results, and analysis.

Chapter 5: Discussion

Discusses findings, limitations, and implications of the results.

Chapter 6: Conclusion and Future Work

Summarizes the work and suggests directions for future research.

Chapter 2

Literature Review

2.1. Biosensors

Biosensors are employed in applications such as disease monitoring, drug discovery, and detection of pollutants, disease-causing micro-organisms and markers that are indicators of a disease in bodily fluids (blood, urine, saliva, sweat). [2]

2.1.1. Background on Biomarkers

A biomarker is an objective measure that that gives an indication of the biological processes happening inside the body at a given moment. [3] They are physical substances found in the body that can be measured. The concentration of biomarkers differs between healthy individuals and individuals with diseases, thereby aiding in diagnosis and monitoring of diseases. [4] Some biomarkers are easy to measure (such as blood pressure, body weight, etc.) while others require tests of blood, urine or tissue samples. [3]

This project will focus on the detection of biomarkers found in blood samples such as the CA-19 biomarker used for pancreatic cancer detection. Note: Verduidelik bietjie oor CA-19. The concentration of these biomarkers in blood can give an indication of the presence and progression of a variety of diseases, including many types of cancer. [5]

2.1.2. Types of Biosensors

A biosensor consists of an analyte, a bioreceptor and a transducer mechanism combined with the electronics needed to process the signal. [2] The analyte is the substance of interest (such as biomarkers) that needs detection. Bioreceptors are molecules such as enzymes, cells, DNA or antibodies that specifically recognise the analyte. These bioreceptors produce a signal (in the form of light, heat, pH, charge or mass change, etc.) when they interact with the analyte. [2] Antibody based biosensors are the type of biosensor that will be used to detect biomarkers in this project.

Antibodies are produced by vertebrates as part of their immune response to foreign organisms or substances (called antigens). They are the most common biorecognition element used in biosensors. [6] Antibodies are Y-shaped cells that can be divided into two

distinct regions. The top of the Y is variable and binds to a specific antigen depending on the amino acids present in this region. The amino acids present in the constant region (the bottom of the Y) is similar between different classes of antibodies (within the same species of animal). [6] This constant region binds to the substrate of the biosensor during immobilization, leaving the variable region free to bind with antigens. [7]

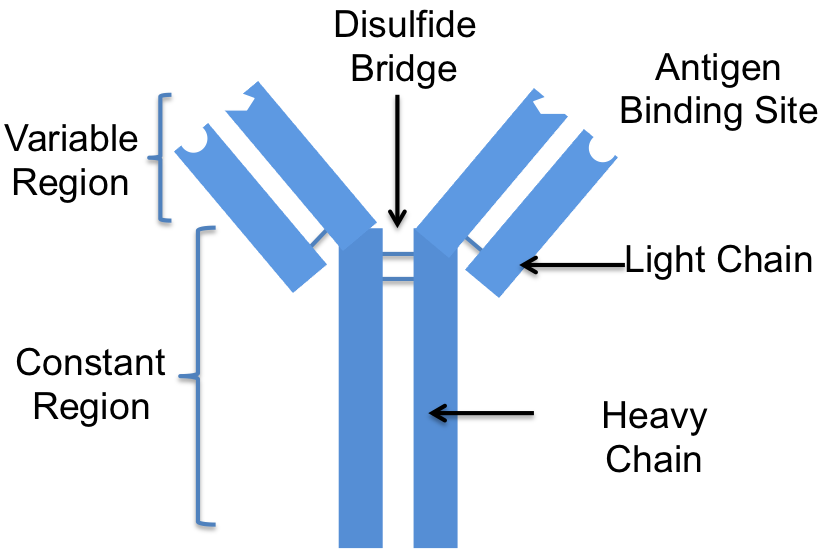

Figure 2.1: Example figure inserted using a LaTeX Workshop snippet.

2.1.3. Transducer Mechanisms

The number of biological binding events indicates the concentration of the analyte in the sample. In order to convert the bio-recognition event into a measurable signal, a transducer mechanism is needed [2]. There are various types of transducer mechanisms that can be used in biosensors, including optical, piezoelectric and electrochemical transducers. This project will focus on biosensors where binding events change the electrical properties of the biosensor, specifically the complex impedance. Thus, electrochemical transducers are of interest.

Electrochemical transducers can use various analysis techniques. In potentiometric analysis, the potential of an electrode is measured against a reference electrode at zero-current [8]. Coulometry applies a constant potential (with regards to a reference electrode) onto an electrode surface to carry out exhaustive electrolysis of an analyte [8]. Voltammetry involves subjecting the sample to a varying potential at the electrode's surface and measuring the resulting Faradaic current [8]. Finally there is electrochemical impedance spectroscopy (EIS), which measures the complex impedance of an electrochemical system as a function of frequency [8]. EIS is particularly suitable for biosensor applications where biological binding events alter the electrical properties of the electrode-electrolyte interface [9]. This project will thus make use of EIS as the transduction mechanism.

Electrochemical Impedance Spectroscopy

This technique applies a small sinusoidal voltage signal to the biosensor and measures the resulting current response. The complex impedance is calculated as:

$$Z(\omega) = \frac{V(\omega)}{I(\omega)} = Z'(\omega) + jZ''(\omega) \tag{2.1}$$

where $Z'(\omega)$ is the real component representing resistive behaviour and $Z''(\omega)$ is the imaginary component representing capacitive or inductive behaviour. [10]

Faradaic vs Non-Faradaic EIS Sensors

EIS biosensors can be categorized into two main types based on their transduction mechanism: faradaic and non-faradaic sensors. [11]

Faradaic sensors rely on electron transfer reactions at the electrode surface, typically requiring redox-active species in solution. These sensors detect changes in charge transfer resistance (R_{ct}) when biomolecule binding blocks electrode access or alters electron transfer kinetics. The equivalent circuit model for faradaic sensors is the Randles circuit, consisting of solution resistance (R_s) in series with the parallel combination of charge transfer resistance and double-layer capacitance (C_{dl}), plus Warburg impedance (Z_w) representing diffusion processes:

$$Z = R_s + \frac{R_{ct}}{1 + j\omega R_{ct} C_{dl}} + Z_w \tag{2.2}$$

Non-faradaic sensors operate without electron transfer reactions, functioning as capacitive sensors that detect changes in the electrical double layer capacitance. These sensors are particularly advantageous for biomarker detection as they require only simple electrolyte solutions and avoid complications associated with redox probes [12]. The simplified equivalent circuit consists of solution resistance in series with interfacial capacitance:

$$Z = R_s + \frac{1}{j\omega C_{interface}} \tag{2.3}$$

Non-faradaic sensors are capacitive in nature, measuring changes in the dielectric properties at the electrode-electrolyte interface when biomolecules bind to immobilized antibodies. When antigen binding occurs, the capacitance decreases due to the displacement of water molecules and changes in the local dielectric constant according to:

$$C = \frac{\varepsilon_0 \varepsilon_r A}{d} \tag{2.4}$$

where ε_0 is the permittivity of free space, ε_r is the relative permittivity of the medium, A is the electrode area, and d is the effective thickness of the dielectric layer. [13] Discuss nyquist lower vs higher frequency and different effects at different frequencies Insert circuit

models for faradaic and non-faradaic sensors

2.2. Signal Processing

2.2.1. Analogue Electronics

2.2.2. Digital Processing

2.2.3. Calculating Impedance

2.2.4. Extrapolating from Impedance to Concentration

2.3. Interface

2.3.1. ESP32

2.4. Related Works

Chapter 3

Design

3.1. Design Philosophy

The aim of this project is to design a impedance spectroscopy system that can be used in a point-of-care setting. Tim Brown's design thinking principles [14] are thus well suited and were used as the bases for the design process. Brown's approach is characterised by five stages: empathising with the end user, defining a clear problem statement, ideating a wide range of creative ideas, building a quick prototype, and finally testing. These phases are cyclical and allow rapid iteration and learning. This project represents the culmination of this design process, although further iterations of the design process could allow further refinement of the end product.

3.1.1. Understanding the end user

Point-of-care (POC) diagnostic devices are essential for decentralized healthcare, particularly in resource-limited settings such as rural clinics and community health facilities. While EIS has proven to be a powerful technique for biosensing applications, existing solutions present significant barriers to widespread adoption in POC environments.

Commercial impedance analysers such as the PalmSens4 offer exceptional technical capabilities, including frequency ranges from 10 µHz to 1 MHz, high resolution (18-bit), and data analysis features such as circuit modelling [15]. However, these instruments have two critical limitations for POC applications. Firstly, they require substantial technical expertise to interpret EIS data, as users must understand the underlying electrochemical principles to extract meaningful diagnostic information. Secondly, commercial systems like the PalmSens4 are prohibitively expensive, with prices €15,000, making them impractical for widespread deployment in clinics, rural health centres, or low-resource settings.

Previous academic projects have successfully demonstrated low cost impedance spectroscopy devices [16] [17] [18], but similarly require users to manually analyse impedance spectra and possess an understanding of EIS principles to interpret the results. This technical barrier prevents POC personnel from using these devices effectively.

POC healthcare providers require a device that performs impedance-based biosensing but abstracts the complexity away from the user, presenting results in an intuitive way.

Quantitative results, rather than qualitative results are sufficient to indicate the need for referral to a specialist and further testing.

3.1.2. Problem Statement

How can we design a low-cost, easy-to-use impedance analyzer for biosensing applications that eliminates the need for specialized knowledge, enabling point-of-care personnel to test for multiple biomarkers?
This problem statement encapsulates three critical design requirements :

1. Accessibility: The device must process raw impedance measurements and convert them to meaningful outputs, removing the need for users to understand Nyquist plots, equivalent circuit models, or phase angle analysis. Running from a battery and being able to operate without a computer are also necessities in rural settings.

2. Affordability: The total system cost must remain under R4,500 (approximately €220), making it orders of magnitude more affordable than commercial alternatives like the PalmSens4 at €15,000, thereby enabling widespread deployment in resource-limited settings.

3. Multiplexing capability: In resource constrained POC environments, healthcare professionals often face high patient loads. Due to this, multiplexed POC testing is of increasing importance for clinical screening [19]. This allows the screening of multiple analytes with minimal involvement from healthcare professionals.

3.2. Functional Design Overview

The system can be broken up into multiple subsystems, each fulfilling a specific function in order to create a complete device that meets the requirements. On the most basic level the device consists of the devices-under-test (DUTs), an impedance analyser and a user interface. The DUT is the biosensor that interacts with the analyte and whose impedance characteristics change based on the analyte concentration. A multiplexer is used to interface between multiple DUT's and the impedance analyser. The impedance analyser can be further broken down into the power electronics, excitation stage, voltage and current measurement stages and finally the processing that uses an STM32. The user interface is based on an ESP32 and communicates with the impedance analyser through UART.

Breek impedance analyser op in further subsystems Discuss wat ons wil bereik en hoe ons dit gan opbreek. Beskryf hoe die circuit die beginsels van biosensors en EIS in ag moet neem, basies wat dit moet doen, hoekom multiplexing en ja dan hoe daai overall doel in gedeeltes opgebreek word.

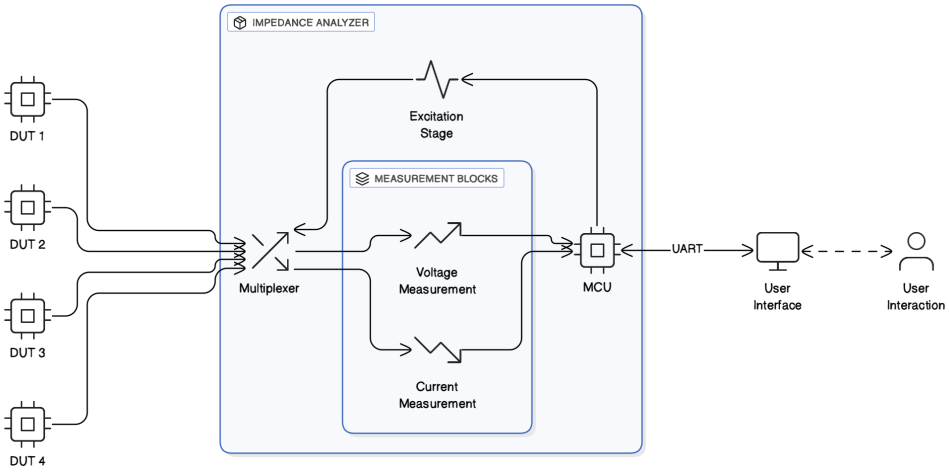

Figure 3.1: System Overview

3.3. Subsystem Design

3.3.1. Excitation

Discuss opsies vir excitation considered: AD5933 chip wat signals generate, DAC ons MCU ander external DAC. Ultimately DAC op MCU. Discuss hoekom sein 10mV moet wees en hoekom ons die volle range van die dac moet gebruik (within die linear region). Finally tie together oor hoekom die excitation phase dus bestaan uit filtering en voltage attenuation Should this be in the literature review?

EIS involves applying a small sinusoidal perturbation to the DUT and measuring the response. This can be either a voltage or current signal, while the other is measured. Generating or measuring voltage is simple as most modern electronics are voltage mode rather than current mode. On the other hand, generating a small current signal is difficult to do accurately and requires circuits such as the improved Howlard current pump. While measuring a current signal also has some complexity, it is significantly easier than generation. It was thus decided that voltage excitation would be used.

Electrochemical impedance spectroscopy (EIS) relies on the system acting as a linear time-invariant system, but most real-world electrochemical systems are inherently nonlinear [20]. To approximate linear behaviour and ensure valid results, EIS uses a small AC excitation signal, typically between 1–10 mVpp [21] [22]. At higher amplitudes, the response deviates from ideal sinusoidal form, causing harmonic distortion and invalid measurements. However, making the excitation too small reduces signal-to-noise ratio, so 10 mVpp is commonly used to balance linearity and measurement quality.

The easiest way of producing a controlled voltage signal is using a digital-to-analogue converter (DAC). Both dedicated DACs and DACs built into microcontroller units (MCUs) were possible options. Figure out hoekom die AD5933 nie sou werk nie (maybe lack of

voltage measurement en discuss TIA issues)

Biosensing requires a bipolar signal with no DC component to avoid charge accumulation at the electrode–electrolyte interface, which leads to polarization. Over time, this would establish a net electrochemical bias, which drives redox reactions and changes the interfacial chemistry of the biosensor. These parasitic reactions alter the impedance characteristics of the electrode, obscuring the true dielectric and charge transfer properties that the EIS measurement aims to quantify. This can be solved by shifting our generated excitation to be biased around ground using a level shifting opamp circuit. However, this also requires providing all analogue circuitry with a negative supply rail. Rather, this project makes use of a buffered virtual ground reference at 1.65V (3.3V/2) and uses this as the midpoint for all the analogue circuitry. This approach ensures that no DC bias is applied to the DUT while negating the need for negative supply rails.

When generating a sinusoidal signal using a DAC, the output is not a smooth analogue waveform, but rather a series of discrete voltage steps. These steps introduce high-frequency components and harmonics that are not present in the original signal. If these unwanted frequencies are not removed, they can interfere with downstream analogue circuitry or be misinterpreted during subsequent analog-to-digital conversion, a phenomenon known as aliasing. To prevent this, an anti-aliasing filter (AA filter), typically a low-pass filter (LPF), is placed after the DAC output to remove high-frequency content and smooth out the signal.

Dynamic range determines the smallest signal the DAC can produce above its noise floor. The theoretical dynamic range (in dB) of a DAC is given by the following formula [23]:

$$\text{Dynamic Range} = 6.02n + 1.76 \tag{3.1}$$

with n being the number of bits of resolution. For the 12-bit DAC found in the STM32F303K8, this results in a dynamic range of 74dB. When designing the AA filter, the dynamic range determines the required level of attenuation at the Nyquist frequency ($f_s/2$). To prevent high-frequency components—introduced by the DAC's discrete step output—from being aliased back into the signal band, the AA filter must attenuate these frequencies sufficiently so that any aliased content falls below the DAC's noise floor, as set by its dynamic range. At the same time, the filter's passband must remain flat at the desired signal bandwidth to avoid distorting the amplitude or phase of the intended output.

If we want to have more calculations insert, the calcs of how to calculate the cutoff of an 8th order raised cosine to have -74dB at fs/2 but it's going to be at least 1/2 page

Due to these requirements, a fixed frequency AA filter is unsuitable when generating a wide range of frequencies from 1Hz-100kHz. Many variable AA filter ICs exist, however many require changing a resistor value to set the cutoff frequency. This would be impractical

when many frequencies are needed. Ultimately the LTC1069 proved to be the only viable option, providing an 8th order lowpass filter that approximates a raised cosine response (with $\alpha = 1$). Importantly it has a cutoff frequency of up to 120kHz (200kHz when using $\pm 5V$ supply rails) set by an external clock and a linear phase response [24]. The clock-tunable nature of the LTC1069 is ideal for this project, allowing easy adjustments through the use of a timer on the STM.

Insert diagrams of stair stepping and frequency components. Dalk sommer van LT Spice

3.3.2. Voltage Measurement

Some impedance analyser designs attempt to infer voltage across the sample by using the known attenuation an applied signal [18]. However, direct measurement of the true voltage across the device under test ensures all sources of non-ideal behaviour (parasitic resistances, stray capacitance, drift, and environmental changes) are accounted for, providing accurate data for impedance calculation. Accurate voltage monitoring is vital because small changes can significantly affect calculated impedance, particularly in low-voltage biosensing circuits.

It is essential to measure the voltage directly across the biosensor, rather than measuring the applied signal only in reference to the virtual ground. This ensures that variations that occur due to the dynamic and non-ideal behaviours at the sensor–electrolyte interface are included, leading to more reliable results.

Two common circuits are used to achieve this: differential op-amps and instrumentation amplifiers. Differential op-amps are simple and cost-effective for basic differential measurements, but they are susceptible to common-mode noise and offset errors [25]. Differential op-amps also have low input impedance, which loads the signal source and can affect test results [25]. Instrumentation amplifiers, on the other hand, are specifically designed for high-precision differential measurement and provide superior common-mode rejection, high input impedance and excellent accuracy even when the input signals are small or operating in a noisy environment [26]. This makes instrumentation amplifiers particularly suitable for biosensing applications where the signals to be measured can be very small, and minimizing interference is critical.

Additionally, it is important to amplify the measured voltage to fully utilise the linear range of the analogue-to-digital converter (ADC), which enhances both sensitivity and resolution. By maximizing the voltage swing within the ADC's input range, the system can discriminate smaller changes in sensor response, thus allowing for better detection of low-concentration analytes.

3.3.3. Current Measurement

The most basic method of measuring current is through making use of a precision shunt resistor. This involves placing a small known resistor in series with the current path and measuring the voltage drop across the resistor. Making use of equation 3.2, the current can be calculated.

$$V_{drop} = I_{in} \times R_{shunt} \tag{3.2}$$

This approach is cheap and easy to implement, but has severe drawbacks. The voltage drop across the resistor impacts the magnitude of the applied perturbation, thereby impacting our measurements. For biosensing, where signal levels are low, even small drops or error sources can affect sensitivity.

Alternatively a transimpedance amplifier (TIA) can be used. A TIA makes use of the following properties of op-amps:

$$V_n \approx V_p \tag{3.3}$$

$$I_n \approx I_p \approx 0 \tag{3.4}$$

Equation 3.3 shows that the positive input (connected to the DUT) is driven to the same potential as the negative input (the virtual ground reference at 1.65V), thereby ensuring a low-impedance path for the current. Conversely, equation 3.4 means that the TIA has a high input-impedance and that the current from the sensor flows entirely through the feedback resistor. The output voltage is then given by:

$$V_{out} = I_{in} \times R_{feedback} \tag{3.5}$$

The feedback resistor can be large without affecting the applied signal, however it does reduce the bandwidth of the TIA. If $R_{feedback}$ is too large, the TIA will experience significant phase shifts and reduced gain at higher frequencies. This can be mitigated by using multiple gain stages.

Biosensing with a fixed voltage perturbation over a wide impedance range means the current can vary from nanoamps (high impedance, low analyte concentration) to milliamps (high impedance, low analyte concentration). This means that using a fixed gain amplifier is impractical. To achieve accurate measurement across this dynamic range, a programmable gain amplifier (PGA) is used to amplify the TIA output and ensure that the output voltage remains within the optimal range for the ADC. The TIA is also designed with multiple gains by switching the feedback resistor values. This prevents saturation for high currents and maximises resolution for low currents.

3.3.4. DUT

The design and manufacture of biosensors are outside the scope of this project. The biosensors described in [16] were used for this project, however the system could easily be adapted to work with other capacitive biosensors. To ensure ease-of-use, a method of interfacing with the biosensors that is simple and reliable needed to be developed. Spring loaded battery connectors were used as they allow the DUT to be easily slid in and out of the device when combined with a 3d printed holder.

Insert 3d model of connectors, dut and 3d print.

3.3.5. Multiplexer

Two approaches towards multiplexing were considered. One approach is to have multiple excitation sources and measurement stages, allowing for simultaneous measurements of all DUTs. This approach has the advantage of speeding up the measurement process, while allowing each DUT to have electronics dedicated to its measurement range. The major disadvantages to this approach is complexity and cost.

Another approach is re-using the same excitation and measurement circuitry, by switching the input and outputs between the DUTs. This reduces the cost and complexity, but is reliant on having a reliable switching mechanism that does not impact the measurements.

Ultimately the best approach was using a single set of excitation and measurement circuitry, multiplexed in order to measure DUTs sequentially. The cost reductions of this approach outweighs the increased measurement time as no user input is required between DUT measurements.

Various options for multiplexers were considered including dedicated analogue multiplexers (MUX ICs), op-amp based multiplexers and relays. Dedicated analog multiplexers consist of a collection of analog switches. They typically use CMOS technology, resulting in compact integration and fast switching speeds. Modern analogue switches are available with very low on-resistance ($< 1\Omega$) and a high degree of flatness [27]. However, leakage currents are inherent to these solid-state devices and can corrupt low-current signals, especially in the nanoampere range [27].

Op-amp-based multiplexers such as seen in Figure 3.2 provide buffering and impedance matching, which make them ideal for multiplexing voltage signals, but the buffering also make them unsuitable for use with current signals.

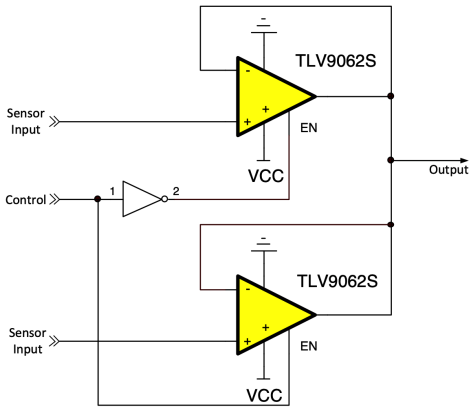

Figure 3.2: Op-amp based multiplexer circuit [1]

Signal relays, in contrast, use electromechanical contacts to physically open or close signal paths, offering near-zero leakage current and extremely low, stable contact resistance that is independent of signal voltage and temperature. This physical isolation and connection ensures that the measured current accurately reflects the biosensor response. While relays are slower to switch and larger than solid-state alternatives, their switching speed is more than sufficient for switching between sensors.

The TXS2-L2-3V DPDT latching signal relay was chosen due to it's small size, low operating current (23.3 mA) and high mechanical lifetime (Minimum 200 000 operations). The major concern of a mechanical relay is the mechanical wear, however at an assumed 2 actuations per measurement and 50 measurements a day, the relays are expected to last more than 5 years. Utilising the DPDT topology of the relay, they can be configured in a tree pattern, allowing for 4 DUT's to be switched using 3 relays as seen in figure **??**.

Despite the low operating current, a driver circuit is still needed to power the relay from a microcontroller GPIO. This consists of a lowside NPN transistor and a flyback diode to protect against voltage spikes when the coil is switched off. The final circuit can be seen in figure **??**

3.3.6. Power Circuitry

With portability in mind, a battery is a requirement for the system. It was decided that the most cost-effective approach would be to utilise a microcontroller with built-in LiPo charging circuitry instead of a dedicated charging circuit as this is commonly available in many ESP32 boards. The 3.3V rail from the ESP will then be used to power the rest of the system.

As mentioned in Section 3.3.1, a 1.65V reference is needed for the analogue circuitry. Due to the small amplitude of the excitation signal, it needs to be highly accurate and stable. This was done through the use of a matched resistor array and a op-amp buffer. Using a resistor array ensures that our reference is the exact midpoint of the supply voltage

despite any tolerances in the precise resistor value, while the op-amp buffers this output to avoid loading the resistor array and causing a voltage drop. Choosing a too large resistor value risks a slightly uneven voltage drop due to the input bias current of the op-amp buffer, on the other hand, a lower value increases the static current draw and power consumption. $1k\Omega$ was chosen as a balance between these tradeoffs.

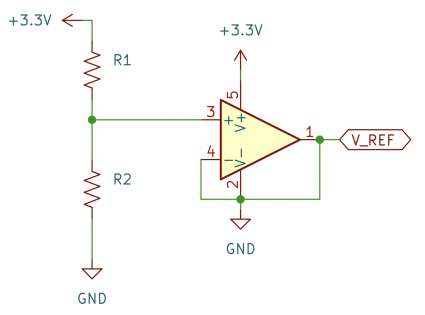

Figure 3.3: Virtual ground reference circuit

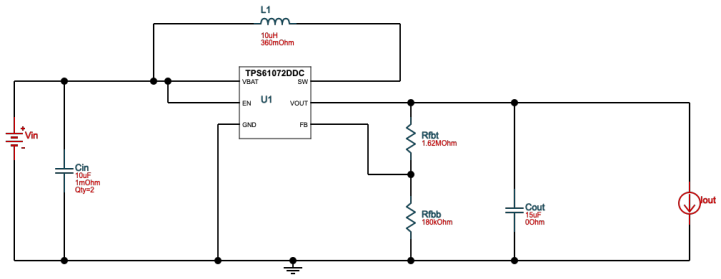

Figure 3.4: 5V boost converter circuit

The LTC1069 AA-Filter requires a 5V supply voltage. A 3.3V to 5V boost circuit was designed around the TPS61072 boost regulator using the TI WeBench power supply design tool [28], ensuring a stable and efficient circuit as seen in figure 3.4.

3.3.7. Signal Processing

3.3.8. User Interface

Fill in once GUI is actually done Talk about flexibility of having both physical buttons and display aswell as web ui, allowing both standalone use and use with either computer, tablet or cell phone, ensuring versatility across environments

3.4. Detailed Design

Gaan meer in diepte oor circuitry maar steeds met generic/ideal coponents. Cover circuitry van elke section, design requirements etc soos 10mV excitation bv. Wat wil ons bereik en dan hoe daai translate na technical requirements en dan die circuitry. Wys LT Spice circuits en sims van elke seksie en combined. Cost is n spec (¡R4500). Refer back to Brown design. Noem dat weens die tight budget and time restrictions van die projek was prototyping gedoen met SPICE en bullshit iets van basiese toetse met esp's en stm's wat rondlê maar anders is as finaal?

3.4.1. DUT

Data van Dr Ebrahim, en dan kry circuit model van daai af.

3.4.2. Voltage Reference

DONE

3.4.3. Excitation Stage

Verduidelik hoekom ons 3.3V output signal van DAC gebruik (maximise range theory). Verduidelik hoekom ons 10mV soek gebasseer op DUT circuit model. Gee brief overview van beginsels van opamp em somme. Verduidelik dan hoekom LPF Anti Aliasing nodig is gebasseer op Frequency domain theory en al daai. Calcs rondom hoe naby aan signal freq cutoff moet wees, dus variable LPF. Te expenny om self te bou dus eerder IC. Was voor en na Filter van DAC sims.

3.4.4. Voltage Measurement

Why is voltage measurement needed when we already know our output voltage. Discuss what to consider when amplifying signal. Discuss LPF en hoekom n volle variable een nie needed is nie (dit is meestal vir noise nie vir anti aliasing van sampling nie, want DAC AA behoort dit te keer).

3.4.5. Current Measurement

Beskryf hoekom current measurement so belangrik is. Wat ons range van currents is. Beskryf beginsels van TIA including somme en circuit analysis. Dan hoekom TIA nie al die amplifications doen nie en why PGA needed is. Raak dan briefly ook op die LPF beginsel selfde as voltage.

3.4.6. Multiplexing

DONE

3.4.7. DSP

Discuss why DSP needed, why microcontroller and not other DSP. Beskryf basies hoe ons filtering gaan doen etc. Die formules en konsepte van freq domain en hoe ons capacitance calc en dan na concentration gaan. Los die details van code en libraries en issues vir Firmware Design section.

3.4.8. User Interface

Baie briefly discuss wat ons vereis van

3.5. Component Selection

Watse komponente ons kies, why en watse aspekte ons consider het en hoekom daai specs belangrik is. How een komponent die ander beinvloed. Wys sims met spesifieke komponent seleksies. Wys calcs vir passive compoinent selections.

3.6. PCB Design

Beskryf filosofie en idees wat mee ingegaa het. Briefly discuss general PCB design principles wat design geguide het (Analogue ground plane etc). Beskryf beperkings van PCB manufactures wat inag geneem moes word (PCB size, layers, trace width via diamtre etc.). Noem briefly hoekom PCB in China eerder as Uni laat maak. Gaan deur design logic en discuss probleem met TIA. Include maybe final PCB diagram en langs dit foto van manufactured PCB.

3.7. Firmware Design

Inculde flow diagram.

3.7.1. ESP

Vertel van wat ons wil bereik en hoekom. Discuss libraries used. Discuss maybe issues rondom C6 en hoe dit gesolve is en hoekom die C6 steeds die rgete keuse was.

3.7.2. STM

Probleme met arm library te groot. Discuss met flow chart hoe DMA en als met DAC en ADC interact en dan UART en badies program flow. Delve into limits van STM en maybe briefly setup van DAC en ADC.

Chapter 4

Testing & Validation

4.1. PCB Testing

4.1.1. Voltage Reference Load Testing

4.1.2. Excitation Stage Testing

4.1.3. Voltage Measurement Testing

4.1.4. Current Measurement Testing

4.1.5. Multiplexing Testing

4.1.6. Complete System Test Without & With MCU

4.2. Validation

4.2.1. Test on Calibration Cell

4.2.2. Test on Saline Solution

4.2.3. Discussion of Results

Chapter 5

Summary and Conclusion

Bibliography

[1] "Sboa311a."

[2] N. Bhalla, P. Jolly, N. Formisano, and P. Estrela, "Introduction to biosensors," *Essays in Biochemistry*, vol. 60, no. 1, pp. 1–1, Jun. 2016.

[3] "Biomarkers | National Institute of Environmental Health Sciences."

[4] A. Rosenzweig, "What Are Pancreatic Cancer Biomarkers? - Pancreatic Cancer Action Network," 2018.

[5] J. A. Ribeiro and P. A. Jorge, "Applications of electrochemical impedance spectroscopy in disease diagnosis—A review," *Sensors and Actuators Reports*, vol. 8, pp. 100 205–100 205, Dec. 2024.

[6] X. Zeng, Z. Shen, and R. Mernaugh, "Recombinant antibodies and their use in biosensors," *Analytical and bioanalytical chemistry*, vol. 402, no. 10, pp. 3027–3038, Apr. 2012.

[7] S. Sueda, "Antibody immobilization for immunosensing," *Analytical Sciences*, vol. 38, no. 1, pp. 1–2, Jan. 2022.

[8] H. S. Magar, R. Y. A. Hassan, and A. Mulchandani, "Electrochemical Impedance Spectroscopy (EIS): Principles, Construction, and Biosensing Applications," *Sensors*, vol. 21, no. 19, p. 6578, Jan. 2021.

[9] J. S. Daniels and N. Pourmand, "Label-Free Impedance Biosensors: Opportunities and Challenges," *Electroanalysis*, vol. 19, no. 12, pp. 1239–1257, 2007.

[10] M. E. Orazem and B. Tribollet, "A tutorial on electrochemical impedance spectroscopy," *ChemTexts*, vol. 6, no. 2, p. 12, Jun. 2020.

[11] R. A. Dorledo De Faria, L. G. Dias Heneine, T. Matencio, and Y. Messaddeq, "Faradaic and non-faradaic electrochemical impedance spectroscopy as transduction techniques for sensing applications," *International Journal of Biosensors & Bioelectronics*, vol. 5, no. 1, Feb. 2019.

[12] L. Huang, C. Zhang, R. Ye, B. Yan, X. Zhou, W. Xu, and J. Guo, "Capacitive biosensors for label-free and ultrasensitive detection of biomarkers," *Talanta*, vol. 266, p. 124951, Jan. 2024.

[13] 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.

[14] T. Brown, "Design Thinking," *Harvard Business Review*, Jul. 2008.

[15] "PalmSens4," https://www.palmsens.com/product/palmsens4/.

[16] T. Ebrahim, "The development of a biosensor for the early detection of pancreatic cancer," 2023.

[17] A. Al-Ali, A. Elwakil, A. Ahmad, and B. Maundy, "Design of a Portable Low-Cost Impedance Analyzer," *BIODEVICES 2017 - 10th International Conference on Biomedical Electronics and Devices, Proceedings; Part of 10th International Joint Conference on Biomedical Engineering Systems and Technologies, BIOSTEC 2017*, vol. 2, pp. 104–109, Feb. 2017.

[18] L. A. Buscaglia, J. P. Carmo, and O. N. Oliveira, "Simple-Z: A Low-Cost Portable Impedance Analyzer," *IEEE Sensors Journal*, vol. 23, no. 21, pp. 26 067–26 074, Nov. 2023.

[19] C. Dincer, R. Bruch, A. Kling, P. S. Dittrich, and G. A. Urban, "Multiplexed Point-of-Care Testing – xPOCT," *Trends in Biotechnology*, vol. 35, no. 8, pp. 728–742, Aug. 2017.

[20] "Electrochemical Measurements: Electrochemical Impedance Spectroscopy | Nanoscience Instruments."

[21] "EIS Quality Indicators and Linearity Correction."

[22] A. C. Lazanas and M. I. Prodromidis, "Erratum: Electrochemical Impedance Spectroscopy-A Tutorial (ACS Measurement Science Au (2023) 3:3 (162-193) DOI: 10.1021/acsmeasuresciau.2c00070)," *ACS Measurement Science Au*, vol. 2023, no. 3, pp. 14–14, 2025.

[23] L. Gaddy and H. Kawai, "DYNAMIC PERFORMANCE TESTING OF DIGITAL AUDIO D/A CONVERTERS."

[24] "LTC1069-7CS8#PBF," https://www.digikey.co.za/en/products/detail/analog-devices-inc/LTC1069-7CS8-PBF/889060.

[25] e. technology, "What are the Drawbacks of Differential Amplifier Circuits?" https://www.eimtechnology.com/blogs/articles/what-are-the-drawbacks-of-differential-amplifier-circuits, Apr. 2024.

[26] "The Instrumentation Amplifier | Operational Amplifiers | Electronics Textbook," https://www.allaboutcircuits.com/textbook/semiconductors/chpt-8/the-instrumentation-amplifier/.

[27] "Selecting the Right CMOS Analog Switch | Analog Devices," https://www.analog.com/en/resources/design-notes/selecting-the-right-cmos-analog-switch.html.

[28] "WEBENCH-CIRCUIT-DESIGNER Design tool | TI.com," https://www.ti.com/tool/WEBENCH-CIRCUIT-DESIGNER.

Appendix A

Project Planning Schedule

This is an appendix.

Appendix B

Outcomes Compliance

This is another appendix.