

Development and implementation of miniature impedimetric systems for biosensor readout

Proefschrift voorgelegd tot het behalen van de graad van doctor in de industriële ingenieurswetenschappen, te verdedigen door

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Acknowledgements

During the course of my PhD I had the pleasure to work together with people of varying professional backgrounds and scientific experience. I would like to take the opportunity to thank everybody I worked with during these years. The first person I would like to thank is my promotor, prof.dr. Ward De Ceuninck. He taught me to work in a very goal-oriented way and interpret results in a critical fashion. My work in his research group started as early as my bachelor internships and I enjoyed being a part of his team ever since.

Also my copromotor, dr.ir. Ronald Thoelen, deserves a special place in these acknowledgements. Always in for good ideas, motivating discussions, supporting conversations, ...

Prof.dr. Patrick Wagner, thank you for giving me crash-courses in biosensors and being available whenever I needed help with anything biosensor related.

My closest colleagues of the ELPHYC group, Dieter and Stijn, made every day at the IMO an enjoyment. Failed measurements, technical problems and other difficulties were often solved by working closely together, accompanied with the necessary sarcastic humor and heated debates. During the years I spent at IMO I also had the great pleasure to work with several bachelor and masterstudents. The input of each and every one of these students is deeply appreciated. Furthermore, I owe special thanks to Wouter Vanaken for continuous technical support and coming up with designs that really helped shape this thesis.

I want to thank everybody from the BIOS group for the constant production of biosensor samples in all possible shapes and layouts and the relaxed atmosphere in the lab and on our conferences. Technical discussions and support from the XIOS EMAP research group is also greatly acknowledged.

For technical questions I could always rely on the expertise of Johnny, Jan and Lieven. Furthermore I would like to thank the administrative staff for helping me with the necessary paperwork and Christel and Hilde for keeping the laboratories a safe place to work.

Last but not least I want to thank my parents for being there for me and giving moral support whenever I needed it.

Abstract

Research in the field of biosensors, i.e. sensors that are based on a biological recognition layer, has steadily increased in popularity over the last few decades. Sensors suited for a wide variety of bio-medically relevant target molecules are constantly being developed. The number of biosensor devices that actually reach the commercial market is however highly limited. Very few examples of such devices can be found, aside from the well-known glucose sensor and pregnancy test strips.

Recent work has lead to synthetic sensors mimicking biological detection. These molecularly imprinted polymers (MIP) offer significant advantages over biological layers, including robustness and long shelf life, making them theoretically ideal for commercial applications. These sensors would thus benefit greatly from a miniaturized readout not only in the end-applications, but also in the development and characterization phase.

In this work small form factor biosensor readout devices are presented. First an introduction in biosensors and portable applications is given, from which it becomes clear that the current commercial biosensor device market is still in its infancy. The number of readout systems suited for a wide variety of these sensors, either for use in the lab or point-of-care settings, is also highly limited. Impedance spectroscopy was chosen as the measurement technique on which to base device design. This technique has proven its use in the development and characterization stage of numerous biosensors. It not only allows for response monitoring but also enables physical modeling of the sensor cell, e.g. in equivalent electrical circuits. Although impedance analyzers are readily available, a combination of high cost, large size and high excitation signals make them unsuited for the targeted applications. Therefore three devices, each targeting a specific biosensor setting, are presented.

First the design steps taken to develop a unit suited for lab characterization are discussed. This eight-channel device is completely controlled and powered by a connected PC and has peripheral ports available for interfacing with other lab equipment. The frequency spectrum and impedance range are specifically chosen to allow for readout and characterization of numerous types of biosensors and sensor layouts. Care is taken to avoid any interference between the device and the sensor under test.

A second device, incorporating touch screen technology and numerous (wireless) communication protocols is well suited for field use and point-of-care applications. The measurement circuitry is based on the previous device and

specifications are comparable, though the number of channels is reduced to four. Bluetooth protocol allows for short-range wireless control of the system, while Ethernet makes the device well-suited for long-distance use over standard network infrastructure. Data is stored on an on-board SD card and an application-specific user interface can run on the touch-screen display.

Last but not least, a smartphone based readout system is presented in which disposable biosensor strips can be inserted. This could bridge the gap towards consumer applications. The choice was made to use the audio signals of the smartphone itself for impedance measurements, thus highly limiting the required external hardware. This puts however some limitations on the frequency spectrum and impedance range. While the previous devices were intended for readout of a wide variety of sensors, the smartphone-based system is specifically intended and fine-tuned for the readout of MIP sensor strips. The electrodes on these strips were custom made using low-cost screen-printing technology.

Performance of the presented devices is verified on passive components and equivalent circuits of biosensors. Setups typically used for lab characterization of these sensors are described and steps taken to interface the units with the setups are explained, after which verification is done on actual bio(mimetic) sensors. Here a close eye is kept on the viability of the systems for measuring within the complete operating range of the sensors. For adequate readout the measurement errors and noise need to be below the impedance response at the detection limit of the sensor.

The design principles presented in this work could lead to the development of commercially viable biosensor devices and form a concept for the use of biosensors in different applications.

Nederlandse samenvatting

Onderzoek naar biosensoren, dit zijn sensoren op basis van een biologische herkenningslaag, is de laatste decennia sterk gestegen in populariteit. Sensoren geschikt voor de detectie van een breed gamma aan biomedisch relevante doelmoleculen zijn continu in ontwikkeling. Het aantal biosensor gebaseerde toestellen dat effectief een commerciële markt bereikt is echter zeer beperkt. Los van de bekende glucosemeter en zwangerschapsteststrookjes zijn zeer weinig voorbeelden van dergelijke toestellen beschikbaar.

Recent onderzoek heeft geleid naar synthetische sensoren die in staat zijn om biologische detectie na te bootsen. Deze moleculair imprinte polymeren (MIP) bieden voordelen zoals robuustheid en lange levensduur ten opzichte van biologische lagen. Hierdoor is dit type sensor uitermate geschikt voor commerciële toepassingen. Miniatuur uitleesapparatuur kan bijgevolg een groot voordeel bieden in zowel eindapplicaties als de ontwikkelingsfase van deze sensoren.

In deze thesis wordt de ontwikkeling van compacte uitleestoestellen voor biosensoren toegelicht. Eerst wordt een introductie in het onderwerp van biosensoren en compacte toepassingen gegeven, waaruit blijkt dat de markt van commerciële biosensor toestellen nog in de kinderschoenen staat. Ook is er weinig tot geen uitleesapparatuur beschikbaar die geschikt is voor een breed aan biosensoren. Impedantie spectroscopie is gekozen als gamma uitleestechniek waarop de ontwikkeling van de toestellen gebaseerd is. Deze meettechniek heeft zijn nut reeds bewezen voor de ontwikkeling en karakterisering van talloze sensoren. Niet alleen kan de respons van een sensor opgevolgd worden, maar de sensorsetup kan ook volledig fysisch gemodelleerd worden, vb. in equivalente elektrische circuits. Hoewel impedantie analyzers commercieel beschikbaar zijn zorgen combinaties van hoge kostprijs, grote omvang en hoge werkingsspanningen ervoor dat deze toestellen niet geschikt zijn voor biosensor uitlezing. Daarom worden drie systemen voorgesteld die elk focussen op een specifiek toepassingsgebied.

In eerste instantie worden de stappen om tot een systeem te komen geschikt voor laboratoriumgebruik uit de doeken gedaan. Het ontwikkelde acht-kanaals uitleestoestel wordt gebruikt in combinatie met een meetcomputer en is in staat te communiceren met andere labo-apparatuur. Het frequentiespectrum en impedantie bereik zijn specifiek gekozen voor de uitlezing en karakterisering van een breed gamma aan biosensoren en sensor structuren. Er wordt zorg gedragen om invloeden van het uitleestoestel op de geteste sensor te voorkomen. Een tweede toestel dat bediend wordt door middel van een aanraakscherm en verscheidene communicatieprotocollen ter beschikking heeft is uitermate geschikt voor veldwerk en point-of-care toepassingen. Het meetcircuit is gebaseerd op het voorgaand systeem en de specificaties zijn vergelijkbaar. Het aantal kanalen is echter gereduceerd tot vier. Bluetooth protocol laat toe het toestel over korte afstand draadloos te bedienen terwijl Ethernet langeafstand communicatie verzorgd via standaard netwerk infrastructuur. Data wordt opgeslagen op een SD-kaart en een applicatie-specifieke gebruikersinterface kan op het aanraakscherm worden weergegeven.

Verder wordt een smartphone-gebaseerde oplossing voorgesteld voor de uitlezing van wegwerpbare sensor strookjes. Op deze manier wordt een brug gevormd naar consumenten toepassingen. Er is geopteerd om de audio signalen van de smartphone te gebruiken voor impedantiemetingen, waardoor de benodigde externe hardware sterk beperkt blijft. Dit heeft als gevolg dat het frequentiespectrum en het impedantiebereik relatief klein zijn. De voorgaande toestellen laten toe een breed gamma aan biosensoren uit te lezen. Het smartphone-gebaseerde systeem is daarentegen specifiek ontwikkeld voor de uitlezing van MIP sensorstrookjes. De elektrodes op deze strookjes worden gefabriceerd door zeefdruktechnologie.

De presentaties van de ontwikkelde systemen wordt geverifieerd aan de hand van passieve componenten en equivalente circuits van biosensoren. Meetopstelling die typisch gebruikt worden voor karakterisering van sensoren in het lab worden toegelicht en stappen die nodig zijn om de toestellen te interfasen met deze setups worden uit de doeken gedaan, waarna verificatie gebeurt op bio(mimetische)sensoren. Hierbij wordt aandacht besteed aan de mogelijkheden van de toestellen om biosensoren uit te lezen in hun volledig werkingsgebied. Hierbij is het belangrijk dat meetfouten en ruis lager zijn dan de impedantie verandering bij de detectielimiet van de sensoren.

De ontwerp principes gepresenteerd in deze thesis kunnen leiden tot de ontwikkeling van commercieel bruikbare biosensorsystemen en vormen een concept voor het gebruik van biosensoren in diverse toepassingen. Innovation distinguishes between a leader and a follower

- Steve Jobs

Chapter 1

Introduction to hand-held biosensor applications

Many present-day health issues are related to elevated concentrations of specific molecules or gene mutations in the human body [1]. The identification of these biomarkers is a popular field of research, since the detection can lead to accurate, early-stage diagnosis of possibly life-threatening conditions. C-reactive protein (CRP) is a particular example of a molecule that can be used as an indicator for early stages of inflammation in internal organs and cardiovascular issues [2]. Since many diseases are related to or caused by molecules present in the environment, the fast and highly sensitive detection of these molecules can also greatly aid treatment [3]. Ideally one would have a portable device, usable in the field, providing highly sensitive and selective detection of a wide range of bio-medically relevant molecules. Biosensors can provide a possible outcome on the sensor side of these systems.

During previous centuries canaries were used in coalmines as a means of detecting lethal carbon monoxide (CO) and methane gas (CH_4) . These birds, which produce a characteristic fluting sound, are much more sensitive to these gasses than humans and will thus be rendered unconscious and die much faster upon exposure. As long as the bird kept singing, miners would know there was little risk of asphyxiation or gas poisoning. The miners thus used a means of biological recognition (the canary), together with a transducer (the singing sound of the canary) and a readout mechanism (the miner's hearing) to detect the presence of specific gasses. This can be seen as the first widespread biosensor, while "a canary in a coalmine" has become a general expression for a warning of things to come.

Over the years these canaries were replaced by specialized measurement equipment. Handheld devices became available which allow for fast, precise readout of selected gasses, increasing security for the miners. Although a large part of the coalmines have closed, these sensors are still present. Fairly recently a system for the detection of airborne pathogens, e.g. the infamous anthrax, was presented, even carrying the name "Canary" [4]. It is a general trend in any sensor system to work towards size and cost reduction while aiming at higher speed and wider detection range. This is especially true when working towards broadly applicable biosensing systems. Examples of commercially available biosensor-based devices are glucose sensors, used by diabetes patients, and the well-known pregnancy tests [5, 6]. This introductory chapter gives an overview of biosensing systems, starting from the basic biosensor layout. Since the focus of this work lies on the development of miniature impedimetric readout systems, a brief overview of the current market of portable biosensing systems is given in the second paragraph. The concluding third paragraph of this chapter explains the aim of the conducted study.

1.1 Biosensors

A sensor is defined as a system that converts a physical quantity into a readable signal, as can be seen in figure 1-1 [7]. The physical quantity is detected by a sensing element, which gives a physical response. This response is most often converted into an electrical signal by means of a transducer. Signal processing allows for output to a user, e.g. by means of a display or data storage.



Figure 1-1: General layout of a sensor system

Biosensors follow this basic layout and differ only from other types of sensors by the use of a biological sensing layer. Most literature describes a biosensor as a biological recognition layer, placed on an immobilization platform, together with a transducer enabling some way of readout [8]. Figure 1-2 illustrates this structure.



Figure 1-2: Basic biosensor layout

In general, the sensing layer is able to bind specific target molecules present in an analyte. This molecular detection results in chemical or physical changes in the sensing layer, which are translated by the transducer into a measurable electrical signal. Several readout techniques exist to translate this response in a human-readable signal to allow for data interpretation.

The concept of these modern day biosensors was established in 1962 with Clark's design of an amperometric glycose oxidase electrode [9]. This sensor detected the amount of oxygen present in a liquid, a parameter which is directly proportional to the glucose concentration. The principle of enzyme-based electrodes was further developed by, among others, Updike and Hicks [10]. Almost fifteen years passed before this measurement principle was actually used in a commercial device [11]. Breakthroughs were later achieved in transducer principles and readout techniques for these sensors, with notable advances including thermal and optic sensing. These glucose-sensing devices evolved since then towards compact consumer-friendly tools, mainly for use by diabetes patients.

During the 1980s new sensing elements were utilized, the most famous being immunosensors based on antibody-antigen binding [12]. These sensors were already described in literature in the early 1970s, but it wasn't until the early 1990s that commercial devices were based on this immunosensing principle [13]. Development of new sensing mechanisms and readout techniques continued during the subsequent decades. The general principle of these sensors remained however the same.

1.1.1 Sensing elements

Classification of biosensor types is commonly done based on the sensing layer used. As was already briefly addressed, the first commercially available biosensing devices were based on enzymatic interaction. Enzymes are large, protein-based molecules, which can be seen as catalysts for a certain chemical reaction. This catalysation principle enables the process of decomposing a molecule into sub-entities. As was shown by Fischer in 1894, enzymatic binding to the target molecule is highly specific [14]. Furthermore, the enzymes themselves are not consumed in the reaction, increasing re-usability of the sensor. In sensor design the enzymes can be for example trapped in membranes, a mechanism commonly described as microencapsulation. These membranes can subsequently attach to an electrode, forming a stable reliable structure that is permeable for the analyte and enabling readout. Discussion has risen whether enzyme-based biosensors are selective enough to be used on a wide variety of practical, field-obtained samples [15]. Nevertheless, aside from the glucose sensor devices, several practical applications can be found, for example in the detection of penicillin [16, 17].

Like enzymes, immunosensors are also based on proteins. Antibodies are the best known and most used type of immunoreceptors. An antibody (Ab), or immunoglobin, is a Y-shaped highly selective protein, which is able to recognize and bind to a specific antigen (Ag). The tips of the Ab contain paratopes for specific binding to an Ag's epitope, allowing for a lock-key type coupling. This binding either allows the Ab to neutralize its target or mark it for destruction by other parts of the immune system. One sensor application based on Ab-Ag binding is the famous enzyme-linked immunosorbent essay (ELISA)-test, for example used for C-reactive protein (CRP) screening [18]. In this test method the Ag's are immobilized on a substrate, e.g. by means of primary Ab's present on this substrate. Secondary Ab's, labeled with a fluorescent label, bind to this immobilized Ag's, resulting in detectable color changes. Figure 1-3 illustrates this test principle.



Figure 1-3: ELISA test principle [19]

Immunosensors have been used in numerous biosensor applications, e.g. in devices used for saliva testing [20]. A downside of the use of Ab's in biosensor application is the instability of the proteins which causes difficulties in immobilization. Therefore, research is being conducted towards smaller, Ab-like, molecules with similar functions [21]. Another issue is the difficulty to undo the Ab-Ag binding for sensor reuse [8].

Nucleic acid mutation sensors are one other important type of biosensors, since deoxyribonucleic acids (DNA) are considered to be essential building blocks for all known forms of life. DNA consists of two long polymer chains containing nucleotides. These nucleotides can be four base types: adenine (A), thymine (T), guanine (G) and cytosine (C), of which A can bind with T and G can bind with C. This interacting results in the formation of two and three hydrogen bonds for A-T and C-G respectively. When this binding occurs, thus linking the two polymer strands together, repulsion from negative charges in these strand needs to be surmounted [22]. This makes for relatively weak bindings, which can be undone. When mismatches are present in the strands, this hybridization process will occur less likely and binding between the strands is weaker. Furthermore a single nucleotide mismatch (SNP), illustrated in figure 1-4 (left) [23], present in the large DNA duplexes can already cause genetic diseases.



Figure 1-4: SNP (left) and fluorescent DNA microarray (right)

These SNPs can be detected in DNA assay measurements. The measurements are commonly conducted on a solid surface containing single stranded probe DNA. When complementary strands are introduced, hybridization occurs, resulting in readable electrical and optical change. These measurements are usually performed in microarrays, where a large number of probe DNA sequences is present. When a solution containing fluorescently labeled target DNA sequences is added, the amount of probe-target complex can be read out by means of optical methods, as illustrated in figure 1-4 (right) [24]. This allows for characterization of DNA in research and even commercial applications [25]. Due to the large array size it is possible to characterize the whole human genome in a single measurement. Although this is a reliable, widely used technique, there are several downsides. The measurement time is mostly determined by the required preparation, which can run up to tens of hours. This method is also an end-point technique and fails to provide information about the hybridization dynamics [26].

In recent developments, SNPs could be detected in both hybridization and denaturation measurements using impedance spectroscopy as a measurement technique [27]. It was shown that denaturation causes a significant drop in impedance. This technique was further fine-tuned, allowing for NaOH-induced denaturation measurements by monitoring the time constant of signal changes [28]. This showed that the denaturation time is directly related to the binding strength of the two strands, and thus related to the presence of SNPs. Another recently developed SNP detection technique is based on the thermal resistivity of DNA samples. As was shown in [29], hybridized DNA has negligible heat resistivity. However, when denaturation occurs, a significant increase in this heat resistivity is shown. This principle could prove useful in temperature based DNA denaturation monitoring. Furthermore, since denaturation can also be thermally induced [30], temperature-based denaturation setups could allow for small scale, simple measurement devices.

The majority of cell-based sensors are based on the use of micro-organisms which provide detectable responses to environmental factors, e.g. exposure to a specific analyte. As cells inhibit a natural behavior to attach to surfaces they are relatively easy to immobilize on sensor substrates. Though research is being conducted towards single-cell biosensors, the majority of the present day lab setups are based on monitoring large populations with sizes reaching thousands of cells [31, 32]. The cells are commonly seeded in well plates containing a medium which provides a stable environment and nutrient supply. When analyte is added to certain wells a response can be seen in the behavior of the cells and in the population size. Though this response is most often read in optical ways or even manually with a hemocytometer, recent developments have shown the possibilities for electronic monitoring of cell populations [33, 34].

The discussed sensor principles have proven useful in several applications, but share common challenges when it comes to field usability. Since these sensors are based on biological layers, the shelf life is rather limited. Production cost can be high and laboratory animals are often needed for acquisition of biological molecules. Also, care should be taken in respect to exposing these biological layers to various measurement conditions, e.g. varying temperatures and different analytes. Molecular imprinting can provide an outcome for these problems. A molecularly imprinted polymer (MIP) is a synthetic material with the ability to mimic molecular recognition occurring in natural receptors. MIPs are stable in many environments, are relatively low-cost to produce and have a long shelf life [35, 36]. MIP particles can be produced by allowing a template molecule, i.e. the target molecule of the synthetic receptor, to form polymerization complexes with functional monomers. These complexes are formed by non-covalent interactions, e.g. hydrogen bonds and Van der Waals forces between the monomers and the template molecule. When a highly crosslinked polymer matrix is formed around the complexes, a rigid structure is obtained. If the non-covalent bonds are broken the template molecule can be removed from the matrix. This results in shape and functional specific cavities in which the target molecule can bind.



Figure 1-5: MIP fabrication process: template molecule with functional monomers, polymerization complex, polymer matrix and sensing cavity [37]

The fabrication steps are illustrated in figure 1-5. Non-imprinted polymers (NIPs) are usually produced to function as a reference during measurements. These NIPs are produced following the same protocol, though without addition of the template molecule.

Target molecules, which are to bind in the MIP cavities, need to be able to move through the porous polymer matrix. The template molecules used in MIP production thus need to be small in size in order to allow for detection. Another prerequisite is the presence of functional groups on the template molecule for forming non-covalent bonds with the functional monomer. Furthermore any interference from the template molecule on the polymerization process should be avoided [38]. Another challenge in MIP sensor design is the way of immobilizing the sensing particles on a solid substrate. Matrix encapsulation, which is also used on enzyme-based sensors, provides a convenient outcome. A conjugated polymer is first attached to the substrate. The MIP particles are subsequently baked into this layer, resulting in a mechanically stable layer [39, 40]. Target molecules that can be detected by MIP sensors include, but are by far not limited to, L-nicotine [40], histamine [39, 41], serotonin [42], lysozyme [43], adenine [44] and malachite green [45].

1.1.2 Transducers and electronic readout

One other key element of a biosensor is the transducer, as this component is responsible for translating the biochemical binding events into physical changes. This conversion can be done in a number of ways.

Calorimetric biosensors rely on the heat released by the biochemical binding events. The heat transfer depends largely on the enthalpy of the reaction and can be undetectably low for some sensor types. The most commonly used thermal biosensors are based on enzymatic receptors, e.g. glucose [46] and esterase [47], though research is conducted towards thermal MIP-based sensors [48]. Several mass-produced low cost miniature electronic components are readily available for thermal sensing. Thermistors rely on changes in electrical resistance to monitor temperature changes. Resistive thermal devices (RTDs) are based on the changes in resistance of metal foils, e.g. platinum foils in Pt100 sensors. Thermocouples on the other hand are based on the Seebeck effect, where a temperature difference between two positions on a metal causes a voltage. By combining two metals in a hot junction and monitoring the voltage at a cold junction point, temperature can be determined. The change in resistivity or output voltage in these sensors due to temperature changes can be directly quantified using existing electronic measurement equipment such as digital multimeters (DMMs). Handheld DMMs often have dedicated thermocouple connectors together with internal cold junction compensation to allow for handheld temperature readout. This technique is however susceptible to measurement errors due to undesired temperature fluctuations in the sensor system. This is especially the case when measuring sensors with low thermal response, as is common in biosensors.

Anisotropic crystals like quartz inhibit a piezoelectric effect, giving out an electrical signal when mechanically stressed. Likewise, when an electrical signal is applied, mechanical movement can be induced. This way vibrations can be induced by applying an AC signal. Each crystal has its own natural resonance frequency, which is altered when the mass changes, according to the Sauerbrey equation [49]. This principle forms the basis for quartz crystal microbalances (QCMs), a type of micro-gravimetric sensors. If a biological receptor layer is immobilized on electrodes present on a QCM crystal, the reception results in changes in the mass and thus also the resonance frequency, which can be electrically read out. This is a commonly used technique for MIP based sensors [40, 50]. An issue with this technique is the one-channel layout of most QCM crystals. This causes an increase in cost and size, since two crystals are needed for both MIP and NIP readout. The problem has been overcome by the development of multichannel QCM sensors, enabling single crystal differential measurements [51]. Electronic spectrum and network analyzers can be used to read out the shift in resonance frequency upon sensor response. Several handheld spectrum analyzers, typically controlled by a PC are commercially available at relatively low cost. Care should however be taken to rule out noise caused by external sources and the measurement equipment itself. Since the resonance frequency in these types of crystal commonly lies within the MHz range, radio frequency interference can significantly hinder accurate readout. This is especially the case when performing measurements in high radio frequency noise environments, e.g. in hospitals [52].

Label-free optical biosensors typically rely on Raman spectroscopy, interferometry or surface plasmon resonance (SPR), the latter being one of the most recent and advanced methods for DNA biosensor readout. Single stranded DNA can be immobilized on a metal surface. When complementary DNA stands are brought into contact with the sensors hybridization occurs, resulting in a measurable change in refractive index of the sample [53]. Highly specialized and often bulky measurement equipment is needed to perform surface plasmon measurements, although research is being conducted towards miniature SPR systems [54]. The DNA assay shown in figure 1-4 utilizes fluorescent labels, which can be optically detected. The fact that this is not a label-free technique is a significant disadvantage in respect to other techniques discussed here. Since preparation of the receptors or target molecule is needed, the usefulness for invivo applications is highly limited. This is also the case with magnetic biosensing, where the analyte needs to be treated with magnetic beads [55].

The term electrochemical readout encompasses a number of readout techniques. Potentiometric systems rely on changes in the potential between two electrodes. Senor particles can be immobilized on an ion-selective indicator or working electrode, while the potential is measured in respect to a chemically inert counter electrode. This technique is for example used in glucose sensors, where enzymes react with an analyte and the working electrode senses the resulting release of ions. The enzymes are most often placed on this working electrode and sealed by a semi-permeable membrane. Readout of a potentiometric sensor cell can be done with low-cost, portable voltmeters. Voltametric sensors on the other hand measure the current while an external voltage is applied to the measurement cell. In amperometry a constant voltage, high enough to bring about oxidation and reduction reactions, is applied to the cell. In the other variant of this technique, cyclic voltammetry, voltage ramps are applied to enable electrochemical reactions. The famous Clark oxygen electrode is based on this measurement technique. In both cases the resulting current can be measured using a DMM, though specific equipment is needed to perform voltage sweeps. Electrochemical impedance spectroscopy (EIS) involves frequency dependent monitoring of changes in complex impedance and has been used as a readout technique for a large number of sensors. Physical information can be determined about the electrochemical measurement cell and the receptor layer itself using equivalent circuit modeling. Since this is the main technique used in this work, impedance spectroscopy is discussed in-depth in the next chapter.

1.2 Portable biosensor systems

Numerous readout devices are available which utilize the discussed measurement techniques. Most commercially available systems are however only intended for lab use and the use of these specific techniques in field devices or hand-held units is rare.

Glucose sensors were the first biosensors to be developed into complete, miniature devices intended for the consumer market. This was mainly due to the commercial relevance and developments in sensing mechanisms. The first commercially available glucose monitors, the 1970's Ames Reflectance Meter shown in figure 1-7 (left), used reflectometry to read out Dextrostix test strips. These test strips were already commercialized in 1965 and relied on visible color change when glucose-containing blood was applied. The Reflectance Meter was in essence a photoelectric cell coupled to a readout needle. A constant light source was present in the device and the blue colored test strip would reflect light proportional to its clarity, which in turn was proportional to the glucose level [56]. Readout accuracy was dramatically improved by using this device since factors such as external light sources and user interpretation of test strip color were ruled out.

With the principle of enzyme-based glucose biosensors came the development of compact amperometric readout techniques. A voltage is applied over a working electrode on which glucose-oxidizing enzymes are immobilized and a counter electrode present on a test strip. When glucose-containing blood is placed on this test strips a catalysation reaction occurs towards gluconolactone. The resulting change in electrical current, proportional to the glucose level present in the applied blood drop, can be easily quantified. Development of this electronic readout technique eventually lead to miniature glucose monitoring devices incorporating almost exclusively digital technology and LCD displays like the OneTouch UltraEasy shown in figure 1-7 (right) [5].

Figure 1-8 shows a more recent example of a commercial hand-held biosensor application. The Philips Magnotech, depicted in the left image, is a protein sensor using cartridges containing magnetic particles with a diameter of hundreds of nanometer, which are coated with ligand molecules. A measurement chamber inside the cartridges in filled with 1 μ l of an applied droplet of blood by means of capillary forces. The ligand molecules bind with target molecules present in the blood. When a magnetic field is activated the magnetic beads, and thus the attached target molecules, are drawn towards an active layer. A secondary magnet allows for removal of beads containing solely unbound ligands. Optical reflectometry is used to detect the presence of particles and thus the attached proteins on the active layer, as is depicted in figure 1-8 (right) [57, 58].



Figure 1-7: Ames (left) and OneTouch (right) glucose meters

The use of disposable cartridges can greatly increase the application domains of biosensor readout devices. Medically relevant concentrations of target molecules can be detected in blood plasma within minutes [59]. The readout mechanism used to quantize biosensor response is however rather complex when compared to electrochemical readout as used in the present-day glucose sensors.



Figure 1-8: Philips Magnotech device (left) en optical readout method (right)

When looking at the previous examples it becomes clear that it is possible to use electrochemical readout for specific sensor strips, as is the case in the

glucose sensors, or to use highly complex readout mechanisms in general measurement devices. Impedance spectroscopy combines the advantages of electrochemical readout with the possibility to analyze the physical properties of a complete biosensor measurement cell. Impedimetric readout of biosensors in the lab is usually performed using generic measurement equipment, not finetuned for biosensing. Additional equipment is often needed, e.g. multiplexers and signal attenuators, to perform non-invasive multi-channel measurements. Furthermore these devices are either intended for rack-mounted or tabletop use. Even when looking specifically at the commercial electrochemical impedance analyzer market, devices are rarely suited for any use other than in fixed laboratory setups. Figure 1-9 illustrates two examples of electrochemical impedance analyzers, specifically suited for low-frequency, low-voltage biosensor measurements. The eDAQ Z100 is a 16-bit data acquisition device that can be interfaced with the compact EA163 potentiostat to perform impedance measurements within a frequency spectrum of 1 mHz to 100 kHz [60]. The Ivium Technologies IviumStat on the other hand is a general electrochemical readout system enabling impedance spectroscopy in a frequency spectrum of 10 µHz to 8 MHz with excitation voltages as low as 15 μV [61]. Both devices have proven themselves in biosensor readout settings [62-64].

These units are significantly less bulky than generic impedance analyzers and specifically designed towards electrochemical readout. The devices are however not yet fine-tuned for field use and an external PC is needed for complete measurement control and analysis.



Figure 1-9: eDAQ EZR-100 (left) and IVIUM STAT (right) electrochemical impedance analyzers

Recent advances in impedimetric readout techniques include the development of miniature, multichannel devices to enhance the field applicability of biosensors. Most of these systems are targeted for a specific type of biosensor or a specific application. Examples include an analyzer fine-tuned for quartz crystal

resonating sensors [65] and a device based on the Analog Devices AD5933 chip, specifically targeted towards applications in functional textiles [66].

One other popular application of impedance spectroscopy can be found in heart rate monitoring. Impedance cardiography (ICG) has been under development since the 1960s. The main advantage of this technique is that, unlike other techniques like electrocardiograms (EGCs), information is given about the dynamics of the blood flow. Miniature ICG devices are constantly under development, as this is a both scientifically and commercially interesting market.

1.3 The aim of this study

Biosensor development is still an emerging field of research. When looking at the scientific output related to biosensors, as illustrated in the red bars in figure 1-10 one can clearly see a continuing increase in the already high number of publications (source: Science Direct, search term: biosensor). Most of these publications deal with the development of either a new type of sensor or the steps taken to alter an established sensor for other target molecules or for use in different applications. However, as shown in the blue bars in figure 1-10, the number of publications dealing with a field applicable biosensor device is much lower (source: Science Direct, search term: (point-of-care OR embedded OR consumer) AND biosensor).



Figure 1-10: Number of biosensor and biosensor device publications per year



Figure 1-11: The 2009 biosensor market according to Frost & Sullivan [67]

Although this topic has known a steady increase in the last decade, biosensor devices account for only 10 % to 20 % of the total biosensor publications. Furthermore, the number of biosensor devices actually reaching the commercial market is extremely small. Figure 1-11 illustrates the biosensor market in 2009. The graph shows that glucose sensors make up almost one third of the total market share. As was mentioned earlier, glucose sensors were made into basic portable consumer devices already during the 1970s and are continuously being refined due to high demand on the consumer market.

In order for biosensors to penetrate a medical research and consumer market, these sensors need to be incorporated in easy-to-use, compact devices. Such systems are constantly under development for specific types of sensors. It is the authors believe however that the practical applicability, and thus also demand, for biosensors could be significantly increased if universal measurement devices fine-tuned for a broad range of biosensors would be available.

The aim of this work is to develop universal biosensor readout equipment that could significantly increase the field-usability of these sensors. Above all these devices should have no influence on the working principle of the biosensors and achieve high enough resolution to monitor response near the detection limit of the sensor. Furthermore, a close eye needs to be kept on the different markets, e.g. medical laboratories, point-of-care applications and consumer devices. Laboratory equipment benefits greatly from interface options with other lab equipment or PCs for data management. Though this equipment should have relatively small size for each-of-implementation in limited lab space, size is less of a constraint than in point-of-care and consumer settings. These devices

should however be able to multiplex between different sensors. Point-of-care devices on the other hand often focus on compact, portable size and extensive user interfacing options for field use. Last but not least, consumer device benefit form ease-of-use and low cost. In consumer settings the multiplexing of sensors also becomes less of an importance than in laboratories. However, due to unknown factors as vibration, temperature fluctuations and user-induced errors, differential measurements between a sensor and a reference channel are essential.

In this work biosensor readout devices are presented specifically focusing on these markets. A multichannel, miniature, USB-controlled biosensor readout device is presented in chapter 3 that finds its main applications in characterization and lab use of biosensors. A second system, incorporating touch screen technology as well as many communication protocols commonly found in present-day smartphones is specifically suited for field applications and is discussed in the fourth chapter. The fifth chapter illustrates the development of a smartphone based readout system for consumer use.

Aside from explaining the critical designs steps taken in device development performance and applicability are evaluated. To do so, biosensors measurements were performed in specialized measurement setups. These sensors, target molecules and measurement cell are discussed in the second chapter.

Impedance spectroscopy was chosen as the main measurement technique on which to base the device designs. This well established electronic readout technique, based on the complex resistivity of a system, is commonly used for the readout of numerous types of biosensors. However, the general designs principles discussed in this work could also be used in the development of devices based on other readout mechanisms.

Chapter 2

Materials and methods

This chapter describes the materials and methods used in this work. First an overview is given of the chosen biosensor readout technique, EIS, on which device development described in the subsequent chapters is based. Biosensor measurement setups are addressed next, after which an overview of the used sensors and target molecules is given. These sensors are used to verify the performance of the presented devices.

2.1 Electrochemical impedance spectroscopy

As is commonly known, electrical resistance can be defined by Ohm's law as the ratio between an applied direct current (DC) voltage and the current flowing through a circuit. This value is independent of frequency and no phase shift occurs between voltage and current. However, practical circuits and components inhibit more complex behavior, which can be characterized using impedance spectroscopy. Like resistance, impedance expresses the ratio between voltage and current, though measured with alternating current (AC) voltages over a certain frequency range. For each frequency within this range a magnitude (|Z|, $[\Omega]$) and a phase shift (φ , $[^{\circ}]$) can be specified, following the polar notation of formula 2.1 as defined by Kennely in 1893 [68].

$$\mathbf{Z} = |\mathbf{Z}| \sqcup^{\theta} = |\mathbf{Z}| \mathbf{e}^{\mathbf{j} \, \theta} \tag{2.1}$$

Impedance can also be represented in Cartesian form, where it is expressed in a real and an imaginary component. The relationship between both notations is shown in figure 2-1, where Z is a vector under an angle ϕ in respect to a real (R) and an imaginary (X) axis [69].



Figure 2-1: Complex impedance plane

Conversion between polar and Cartesian impedance notation is possible using the standard conversion rules for complex numbers. The methods used to do calculate Cartesian values are illustrated in formula 2.2.

$$Re = |Z|\cos(\theta) \text{ and } X = |Z|\sin(\theta)$$
(2.2)

Likewise, values for polar notation can be calculated using formula 2.3.

$$|\mathbf{Z}| = \sqrt{Re^2 + X^2}$$
 and $\theta = \arctan \frac{X}{R}$ (2.3)

Impedance is usually graphically displayed in either Bode or Nyquist plots, showing the polar and Cartesian values respectively. Bode plots consist of a magnitude plot, expressing the logarithmic magnitude versus logarithmic frequency and a phase plot, expressing the phase shift between voltage and current versus logarithmic frequency. Since Nyquist plots display imaginary versus real impedance, no information can be directly discerned about the frequency. This type of plot is often used in stability assessment of a system.

In circuit analysis a measured impedance is commonly related to a combination of three basic components. An ideal resistors cause no phase shift between applied voltage and resulting current and a constant magnitude is measured over a frequency range. It can thus be concluded that the impedance of a perfect resistor equals the resistance value itself. A capacitor on the other hand behaves in essence as a charge storage device where a voltage is present on plates separated by a dielectric. These components inhibit a behavior that delays changes in voltage and the resulting current is directly proportional to the derivative of voltage over time. Current "through" the capacitor is thus zero when the voltage is at a peak and maximum as the sine of the applied AC voltage crosses zero. The voltage lags 90° on the resulting current, resulting in an impedance phase shift of -90° or $-\pi/2$. Furthermore, the impedance magnitude is inversely proportional to the frequency of the applied signal and the capacitance value (C, [F]), as is illustrated in formula 2.4. This also implies that high-frequency signals can flow through a large capacitor much easier than low-frequency signals through a small capacitor. As this magnitude lies purely in the imaginary plane, it is referred to as reactance. The capacitive behavior can be applied to any element that inhibits charge-storage mechanisms, making it essential in equivalent circuit modeling.

$$Z_{c} = \frac{1}{\omega C} e^{-j\frac{\pi}{2}} = \frac{1}{j\omega C} \quad \text{with } \omega = 2\pi f$$
 (2.4)

In contrast, the current flowing through an inductor lags the applied voltage by 90°, thus resulting in an impedance phase shift of +90°. This is due to the fact that an inductor opposes changes in current by causing a voltage drop in proportion to the rate of current change. The instantaneous voltage over the inductor is thus the highest when the rate of change in current, thus the slope of the current sine wave, is maximal. This occurs at the zero-crossing point of the current. The reactance of an inductor is directly proportional to the frequency of the applied voltage and the inductance (L, [H]), as can be seen in formula 2.5. Current-storage elements present in a circuit can be modeled as inductors, though the occurrence in biosensor modeling is much less frequent than resistive and capacitive elements.

$$Z_{L} = \omega L e^{j\frac{\pi}{2}} = j\omega L$$
(2.5)

In electrochemical impedance spectroscopy (EIS), e.g. as performed on biosensors, very small excitation voltages are used, commonly in the range of several mV. Aside from the influence of voltages on biological receptors and solutions, the main reason for doing so is to maintain linear response. A linear system possesses the property of superposition. The total output of such a

system, caused by different inputs, equals the sum of the separate outputs for each input. This is not the case in electrochemical cells, though pseudo-linear behavior can be seen at low excitation voltages. Linearity is an essential requirement for some signal processing tasks like Laplace transformations. However, even at these low excitation voltages, electrical effects take place in the cell.

Biosensor measurements are usually performed using a conductive analyte, being either a body fluid or liquid inhibiting similar properties as biological liquids, e.g. ionic strength and acidity. The basic equivalent circuit of liquid cell setup, known as simplified Randles cell, forms the starting point for impedimetric biosensor measurement interpretation. Figure 2-2 illustrates this model [70].



Figure 2-2: Randles cell

As was shown in paragraph 1.1.2, the receptor layer is immobilized on a solid substrate. A solid-liquid interface thus exists during measurements. At this interface a translation from electron to ion conduction occurs, resulting in a non-uniform distribution of charges. Ions from the solution will attach to the charged biosensor layer, behaving as an electrode, with a typical separation of 0.5 to 10 nm. This layer is essentially a charged capacitor, consisting of two conductors separated by an insulator, and is commonly known as a double layer capacitance (C_{dl}) [71]. Similar behavior can be seen as previously discussed, i.e. a decreasing impedance with increasing frequency and a phase shift of -90° between voltage and current when measuring this capacitor.

Aside from C_{dl} , a polarization resistance (R_p) is commonly present at the interface. This is due to the polarized electrode (essentially the substrate with biosensing layer), which causes an electrochemical reaction to occur, resulting in a current flow. Furthermore, due to electrochemical reactions at the interface, a charge transfer resistance (R_{ct}) can be formed. This is caused by electrically dissolving of metals when brought into contact with an electrolyte. C_{dl} and $R_p + R_{ct}$ are represented as a parallel circuit.

The analyte itself behaves like a resistive element, following Pouillet's law. Every liquid has its own distinct solution resistivity (ρ , [Ω m]), though it is common practice to express conductivity (k, [Sm⁻¹]) of a liquid instead of resistivity. Using these parameters, the total resistivity and conductivity can be calculated using formula 2.6.

$$R = \rho_A^l \text{ and } k = \frac{l}{RA}$$
 (2.6)

This solution resistance can be measured in series with the parallel $C_{dl}-R_p$ circuit. Other resistive elements, e.g. substrate resistivity and losses in connectors and cabling (R_s), can also be seen as sub-circuits in series with the discussed circuit model.

Since C_{dl} is not strictly a capacitive component but a result of charge distribution, it usually behaves as an imperfect, leaking capacitor. It is common practice in equivalent circuit modeling of electrochemical cells to replace C_{dl} with a constant phase element (CPE). The phase shift is less negative than -90° and, though a decreasing slope can still be seen with increasing frequency, formula 1.4 is no longer correct to use.

Fitting of a measured impedance into equivalent circuit models helps to gain insight in the physical characteristics of an electrochemical cell. Biosensor cells typically consist of a biological receptor layer, which is immobilized on a solid substrate and placed in a liquid. When chemical binding events occur, the interface characteristics and more specifically C_{dl} , change [72, 73]. This modeling technique can thus be used to determine the ideal measurement frequency, i.e. the frequency at which maximum response occurs. This method also forms the basis for capacitance and concap readout devices [74].

2.2 Biosensor measurement setups

Biosensor impedance measurements can be performed in measurement cells with numerous possible layouts. In general a distinction can be made based on electrode structure, the number of channels and the way of adding target molecules. The electrode structure can be either coplanar, where a sensor layer is usually placed on the electrode plane [39, 40], or through-liquid designs, where the sensor layer is placed on top of one electrode while the other electrode is placed inside the electrolyte [28]. The number of channels in

biosensing systems can vary from single channel cells to complete arrays, while the input of liquids can occur from simple addition, i.e. adding droplets, to complex microfluidic setups.

A basic through-liquid, single channel Teflon addition cell was constructed that finds it main purpose in the characterization of sensors in lab setups. Its use in practical applications is highly limited but the design, shown in figure 2-3, allows for fast, temperature stable sensor tests.



Figure 2-3: Single-channel addition setup

Samples are mounted on a copper lid, functioning as measurement electrode, temperature conductor and, together with an O-ring, sealing mechanism for the measurement chamber. This measurement chamber is cylindrical in shape with a diameter of 6 mm and a height of 20 mm, resulting in a maximum volume of 0.57 ml. Type K thermocouples can be inserted in both the liquid and the copper lid to allow for accurate temperature monitoring. Furthermore, a 22 Ω power resistor is present on the copper. This resistor produces a relatively high amount of heat directly proportional to the supplied power, enabling controlled heating of the cell.

A PID-based system was built to allow for precise control of voltages applied over the heating resistor and thus precise control of the temperature. A schematic overview of this unit is shown in figure 2-4. Voltages generated by the thermocouples are read out by an USB-controlled Pico Technology TC-08 data logger with internal temperature reference [75]. A National Instruments USB-9263 analog output module interfaced with an USB-9162 carries module allows for 4-channel, 16-bit low-power voltage output [76]. LM675 OPAMPs, supplied with high power voltages, buffers the voltage of each channel. This
way 4 low voltage, high power outputs are available for connection to power resistors. The system is completely controlled by LabVIEW software.



Figure 2-4: Temperature control unit

Differential measurements between a sensor and a reference sample can, to some extent, help to rule any effects present in a measurement cell aside from specific binding in the sensor layer. A 4-channel measurement cell was constructed, as shown in figure 2-5. As is the case with the cell discussed in the previous paragraph, samples are mounted on copper lids, which use O-rings to seal a measurement chamber. This measurement chamber, with a total volume of 350 μ l, is however shared between the 4 channels and the counter electrode is position at equal distance of 11.5 mm to the samples. Thermocouples are mounted as close as mechanically possible to this counter electrode as well as in each of the 4 copper lids. Technical drawings of the measurement cells can be found in appendix 4.1 and 4.2.

Array-based biosensors allow for the quasi-simultaneous detection of different target molecules, often in a single analyte [77, 78]. Since this technique would enable complete biochemical analysis on very small amounts of liquid, it is becoming a popular topic within biosensor research. There are however several problems which need to be overcome, e.g. chemical crosstalk, to allow for field usable devices [79].



Figure 2-5: 4-channel flow cell

A multiplexer was designed to allow for fast impedimetric readout of 96 biosensor channels in a more practically usable layout. The choice was made to use Roche E-Plate 96 well plates with interdigitated gold electrodes as basis for the design. Due to the standardized size of these well plates the system is compatible with existing lab equipment, greatly increasing usability. Figure 3-12 (left) shows the layout of these electrodes for a single well [80]. A common counter electrode is used for each group of 8 channels, resulting in a total of 12 counter electrodes for the complete well plate. Figure 2-6 (right) shows a cross section of an electrode, with (a) being the gold layer placed on a chrome interface (b) on top of the glass plate (c).



Figure 2-6: Layout (left) and cross-section (right) of the well plate electrodes

The multiplexer, shown in figure 2-7 (left), utilizes Interconnect Devices Inc. spring contacts to interface to the electrodes. These spring contacts can be seen in figure 2-7 (right). The 12 counter electrodes of the E-plate are interconnected inside the multiplexer and channel switching is done by switching between the working electrodes of each well. To avoid any interference between the different wells a switching delay of 100 ms was implemented. This channel switching is done by means of Omron latching relays, controlled by Texas Instruments PCA9353 I²C I/O expanders [81]. This allows for complete control of the multiplexer by a single I²C bus.



Figure 2-7: Multiplexer with a well plate mounted (left) and close-up of the spring contacts used for interfacing with the well plate (right)

A different resistivity of 35 Ω , 55 Ω , 80 Ω or 105 Ω , with ± 5 Ω accuracy, was measured between different wells and the input connector of the multiplexer. This resistivity is dependent of the placement of the wells, with the highest resistivity corresponding to the largest distance between input connector and well. This parasitic component is caused for the largest part by the electrodes on the well plate itself, as it documented by the manufacturer. Correction for these values is done in software.

2.3 Sensors, analyte and target molecules

As was shown in the previous chapter, the already large number of different types of biosensors keeps steadily increasing. This renders device performance analysis on all available biosensor types impossible. Therefore in this work two different sensor types were chosen to illustrate device performance. Synthetic, biomimetic MIP sensors were used which excel in robustness and shelf life. On the other hand the behavior of living biological cells, which are highly sensitive to external parameters, was monitored.

2.3.1 MIP sensors

MIP and NIP particles used in this work were synthesized from a mixture of methacrylic acid (17.8 mmol), ethylene glycol dimethacrylate (36 mmol) and azobisisobutyronitrile (0.60 mmol), following the methods described in [39].

Immobilization of MIP particles on a substrate is often done by first spin coating a polymer backplane layer on the substrate [40, 42]. The particles can then be baked into this layer to ensure adequate surface coverage of the sensor. For this sensor layout a thin film of poly[2-methoxy-5-(3,7-dimethyloctyloxy)-1-4phenylene vinylene] (MDMO-PPV) was spin coated on two 10 mm by 10 mm square aluminum substrates. The MIP and NIP particles were immobilized on these substrates by baking them into the MDMO-PPV layer. Using this technique, a surface coverage of typically 30 – 40 % can be achieved.

Recent research is looking towards alternative approaches for sensor immobilization on a backplane layer. Direct immobilization of sensor particles on various substrates could enable the use of MIP sensors in even more practical applications. By avoiding the need for polymer backplanes, possible side effects, e.g. light-sensitivity and degradation of the MDMO-PPV layer [82], are ruled out.

Thin titanium plates of 10 mm by 10 mm were chosen as sensor substrates for direct MIP immobilization. The main advantage of titanium, aside from being a material of choice for in-vivo applications like implants, is the natural occurrence of oxide layers on the surface. This allows silanization of the surfaces to enable anchoring of the sensor layer. Thorough cleaning of the substrates is required as a first step in this sensor immobilization process. The samples were first placed in an ultrasonic bath filled with isopropanol for 2 min. The cleaned surfaces were then subjected to a solution of 10 % y-MPS in toluene under nitrogen atmosphere for 3 hrs, after which they were rinsed with toluene and ethanol to remove unbound silane moieties. The MIP and NIP mixtures were subsequently introduced to the surface and heated to 65 °C for 10 min to activate polymerization. A top view scanning electron microscope (SEM) picture of the resulting MIP sample, consisting of a solid layer of MIP particles on the Ti substrate, can be seen in figure 2-8 (left). Figure 2-8 (right) shows a side view of the sample with the substrate and a layer of MIP flakes visible.



Figure 2-8: SEM pictures of the MIP layer immobilized on Ti substrate, top (left) and side (right) view of the sample

L-nicotine and histamine were chosen as template molecules in this work and were thus added during MIP synthesis and later removed to leave highly specific binding cavities. The chemical structure of these low-molecular weight molecules can be seen in figure 2-9 [83].



Figure 2-9: L-nicotine (left) and histamine (right) molecules

L-nicotine, with chemical formula $C_{10}H_{14}N_2$, can for example be found in urine with concentrations ranging from 300 nM (non-smokers) to 6.3 μ M (smokers). This molecule is readily available at relatively low cost. Previous research has shown the possibilities for nicotine MIP manufacturing, making it an ideal target molecule for device and sensor verifications [40, 41, 45, 84].

Histamine $(C_5H_9N_3)$ is most commonly generated by mast cells and basophils and plays an important role in allergies [85] and irritable bowel syndrome [86]. Furthermore, bacteria in spoiled food are known to release histamine, making this an interesting target molecule in food industry applications [41, 87]. Similarly to L-nicotine this molecule was previously used as a target in impedimetric MIP sensors [39].

In biomedical readout applications the MIP sensors would be exposed to clinically relevant liquids containing an unknown concentration of target molecules. In this work phosphate-buffered saline (PBS) solution was used to mimic biological conditions. This water-based salt solution contains a mixture of NaCl, Na₃PO₄ and KCl and functions both as a buffer as well as mimicking the osmotic concentrations and ion concentrations of fluids present in the human body. Analytes were prepared by dissolving varying concentrations of target molecule in this buffer solution.

2.3.2 Cell proliferation

The multiplexer described in the previous paragraph and shown in figure 2-7 allows for quasi-simultaneous readout of 96 channels. The miniature size and use of standardized well plates allow for usage in several bio-analytical applications. In this work the multiplexer is used for impedance-based monitoring of biological cell proliferation. Figure 2-10 illustrates the biological cell cycle, were a single cell duplicates its chromosome, resulting in a single cell

with double the amount of DNA [88]. This cell splits into 2 cells during the mitosis phase, which results in a clone of the original cell. This is a continuous process and cells are kept under favorable conditions the total population inhibits exponential growth.



Figure 2-10: Biological cell cycle

Human embryonic kidney (HEK), Chinese hamster ovary (CHO) and microglial BV2 cells are among the most commonly used cell types in biomedical research. These cells have in common that they are easy to grow in vitro and can be used in various research applications. For impedance-based proliferation monitoring murine derived BV-2 cells, CHO cells and HEK293T cells were cultured with Dulbecco's Modified Eagle's Medium (DMEM). The cells were incubated in a humidified incubator (5% CO2) at 37°C and the resulting cell suspension was centrifuged for 10 minutes. The cell pellet was re-suspended in fresh cell growth medium (DMEM). The cells were subsequently cells seeded on the well plate in 100 μ l of DMEM medium.

Chapter 3

A miniature PC-controlled impedance spectroscopy unit

"BioZ°"

The applicability of biosensors in lab setups can be greatly improved by highly accurate, hand-held, multichannel readout systems. An impedimetric readout device requires in its most basic form a means of voltage generation within a set frequency spectrum and readout of resulting current or vice versa. One or more accurate master clock frequencies are required in any system that generates or analyzes signals. Aside from excitation signal conditioning, when a wide range of amplitudes needs to be analyzed a means for splitting this range into subranges is often present. For multichannel readout a way of multiplexing is required and last but not least some way of power supply and user interfacing circuitry needs to be incorporated into the device. The following paragraphs document the development of an EIS readout device specifically fine-tuned for biosensor readout in lab settings.



Figure 3-1: BioZ° block diagram

Figure 3-1 shows a block diagram for the presented unit, which was given the name "BioZ°" for its ability to measure impedance magnitude (Z) and phase (with unit symbol °) of biosensors. The remainder of this chapter explains the design steps taken in each part of the diagram as well as verifying performance of the presented device.

3.1 Digital signal processing

A system suited for impedance analysis generally consists of several components that combine and convert digital and analog signals. Typically a direct digital synthesis (DDS) core is used together with a digital-analog converter (DAC) to generate an analog sine wave at a digitally controlled frequency. This sine wave is passed through a transmit stage, often consisting of amplifiers and buffers, connected to a sample. After amplification and filtering the resulting return signal is passed to a digital signal processor (DSP), which implements an analog-digital converter (ADC), resulting in a digital code. A central processor core is used to control both the DDS and DSP. In these systems careful design of both signal paths and power supply is essential for correct interaction of the separate components. Integration of these systems in a single chip eliminates many of the design constraints associated with noise, interference and signal loss when interfacing digital and analog circuitry. Furthermore it is essential to work towards a single integrated circuit (IC) when miniaturizing equipment.



Figure 3-2: AD5933 block diagram

The Analog Devices AD5933 integrated impedance analyzer provides a possible solution for single-chip impedance spectroscopy units [89]. This high precision impedance converter/network analyzer, with block diagram shown in figure 3-2, uses an internal phase accumulated, 27-bit DDS core coupled to a DAC to generate sine waves at 0.1 Hz frequency precision. The receiving stage consists of a programmable gain amplifier coupled to a 12 bit ADC, whose signal is passed through a DSP core. This DSP performs discrete Fourier transforms (DFT) over 1024 samples, resulting in a 2-complements number for both the magnitude and the phase of the signal. An inter-integrated circuit (I²C) protocol bus provides control to the registers used for setting up parameters and storing results.

It should be noted that the AD5933 itself does not contain memory to store measurement data, nor does it contain any programmable logic. Therefore external processing, e.g. by means of a microcontroller or PC-based, is necessary to control the AD5933 via the aforementioned I²C bus. An evaluation board, shown in figure 3-3, is available as a starting point for circuit design based on this IC [90]. A Cypress CY7C68013A USB microcontroller is used to interface the AD5933 I²C bus to an USB-port [91]. This enables control of the impedance analyzer via supplied Visual Basic software. A 64 kbyte electrically erasable read only memory (EEPROM) is used as temporary storage for program and measurement data. Furthermore the evaluation board has all voltage convertors needed to enable complete power supply via USB-port. Connection to a single sample is made by subminiature SMB coaxial connectors.



Figure 3-3: AD5933 evaluation board

As was demonstrated in [92], it is possible to use this evaluation board as a readout device for bio analytical sensors. However, peripheral circuitry is needed to enable frequency spectrum sweeping and to ensure that a broad impedance range can be read out. This peripheral circuitry was incorporated on a PCB, together with a relay switching multiplexer for multi-channel readout. The resulting system was verified on passive components and a liquid cell used for biosensor readout [93]. This unit can be seen as the starting point for development of the BioZ^o device, although the design was completely modified in the sense of specifications, working principles and component choice to enable miniaturization and to increase performance factors.

3.2 Measurement signal conditioning

An impedance analyzer that is universally applicable in bio analytical setups should support a broad impedance range. The typical impedance at which detection of target molecules is measured depends strongly on the type and layout of sensor layer, substrate and electrodes. This can be seen when incorporating similar sensors on either coplanar [84] or liquid-cell mounted electrodes [42]. Furthermore, the analyte used for the measurements has a significant influence on the characteristic impedance of the sensing system [93]. Aside from the typical impedance of a biosensor setup, significant impedance changes during a single measurement on biological samples are also common [94, 95].

To enable design towards broad impedance ranges, a close understanding of the working principles of the AD5933 chip towards transmitted and received signals is essential. The DDS core inside this IC generates a sine wave at a fixed frequency and voltage. For a 3 V supply voltage this excitation voltage can be either 198 mV, 383 mV, 0.97 V or 1.98 V peak-to-peak, as set up by the user in specific registers. Since the chip is powered solely by positive supply it is unable to handle negative voltages. Therefore a DC bias voltage is always present in the output signals.

Range (register value)	Sine wave (Vp-p)	DC bias (V)
1	1.98	1.48
2	0.97	0.76
3	0.383	0.31
4	0.198	0.173

Table 3-1 Excitation voltages and related DC bias

As illustrated in table 3-1, taken from [89], an increasing sine wave amplitude also results in an increase of this DC bias voltage, resulting in an overall positive voltage.

Both the amplitude of the sine wave as the DC bias voltage can have significant influence on bio analytical measurements. These voltages should be as low as possible to eliminate any effect of signal applied to an electrochemical measurement cell. A downside of lower voltages however is increased susceptibility to noise and interference. An example of voltage-critical applications can be found in living cell sensors or cell proliferation measurements. Typical resting potentials of animal cells lay around 70 mV, with the interior of the cell being negatively polarized in respect to the environment. When this voltage drops, i.e. becomes less negative towards a threshold value, an action potential can be reached. This potential cause triggering of the ion channels, resulting in the release of certain ions [96]. These ions will thus enter the medium during measurements, altering the conductivity and the measured impedance. Furthermore, the release of Ca^{2+} , Cl^{-} and K^{+} ions can have a significant influence on the cell behavior itself [97, 98]. It is therefore important to keep externally applied excitation voltages well below 70 mV.

DC offset voltages can have an influence when dealing with charged particles in the analyte. This is for example the case when varying the pH factor, which causes target molecules to be (de)protonated and thus become more negatively or positively charged, resulting in target molecule movement toward or away from a sensor layer [39, 84].



Figure 3-4: AD5933 output signal conditioning

A non-polar capacitor of 10 μ F was placed in series with the output of the AD5933 to block all DC bias voltages and a simple resistor-potentiometer voltage divider provides an adjustable way for voltage level attenuation. The resulting signal amplitude was set at 60 mVpp. The passive high pass filter that was created in the RC-circuit has a roll off frequency of 4 Hz. The signal is buffered by an operational amplifier (OPAMP) and used as an excitation voltage for the sample. Figure 3-4 illustrates this signal conditioning circuitry added to the output of the AD5933.

The current resulting from the sample impedance needs to be converted to a voltage for analysis in the AD5933. This was done by connecting the sample as an input impedance of an inverting OPAMP circuit (U5), as illustrated in figure 3-5. The gain of this OPAMP is equal to R_{FB}/Z_{sample} . Furthermore, as the AD5933 can only handle positive input voltages, the DC bias voltage removed in the output stage needs to be added. An LM336 2.5 V regulator (D1) ensures a stable voltage of -2.5 V over resistor R8. The gain of the inverting summing amplifier circuit around U6 equals 1 for the output voltage of U5 and 0.6 for the voltage over R8. This result in an addition of -1.5 V DC offset to the signal. Since this circuit forms the last signal stage before entering the AD5933, a highend Texas Instruments OPA627 precision high-speed OPAMP was chosen as U6 [99]. This low input bias current Difet-based component has been known for years as a reliable, low-noise solution for audio-signal amplification, but its frequency response expands well beyond the audio spectrum [100]. The main downside of this component and the reason that it was not implemented in the other OPAMP circuits is its relatively high cost when compared to the other components.

Although the circuit presented in figure 3-5 provides adequate current-tovoltage conversion for analysis in the AD5933, there is one major issue when analyzing a broad impedance range. It becomes clear that, since the input voltage of the amplifier circuit around U5 is fixed at 60 mV, the output voltage of the circuit is determined by the sample impedance magnitude at a certain frequency. As this magnitude can vary strongly during biologic measurements, the amplification factor of U5 will vary accordingly, which may cause the resulting output voltage to become either too small or to go out of range for the AD5933 impedance chip. Since the AD5933 is powered by 3 V, its maximum input voltage lies at 3 V. The internal ADC has a limited resolution of 12 bit, which results in a voltage resolution of 0.7 mV. If the maximum analyzable impedance is set at 1 M Ω , thus corresponding with the 3 V level, the impedance resolution would be as low as 244 Ω . Since a desirable impedance range for the target applications would lie between 10 Ω and 10 M Ω , the circuit is by itself inadequate to perform bio-analytical impedance measurements.



Figure 3-5: Sample current-to-voltage conversion and signal conditioning

This problem was overcome by replacing the fixed feedback resistor by a set of relay-switchable resistors. This way, the range from 10 Ω to 10 M Ω is divided in several sub-ranges, each with 12 bits measurement resolution. The minimal amplification factor of the OPAMP circuit was chosen to be 2 while the resolution of the AD5933 ADC is 0.7 mV. Since the excitation voltage is 60 mV, this results in a minimal, worst-case scenario measurement resolution of 0.7 mV / (2 x 60 mV), or 0.58 %. To obtain this minimal resolution and by building in a safety margin from the absolute maximum voltage a set of five resistors was deemed most suited for measuring the desired impedance range.

Impedance magnitude can be calculated by converting the ratio between excitation and received voltage, referred to as a dimensionless magnitude, and a known gain factor according to equation 3.1.

$$|Z| [\Omega] = \frac{1}{Gainfactor [\Omega^{-1}]. magnitude}$$
(3.1)

The gain factor is a value, set in a file stored on the controlling PC, which is determined by performing a measurement over a known resistor and thus known impedance magnitude. Likewise, the phase shift of each calibration resistor is stored to rule out undesired phase shifts due to parasitic components during measurements. Since a set of five feedback resistors was chosen to divide the impedance range into subranges, five calibration resistors are also needed to calibrate the device for each subrange. However, as most OPAMPs inhibit non-linear behavior to some extent throughout their usable voltage range, each subrange was further divided in calibration ranges. Figure 3-6 illustrates this principle. Here a feedback resistor of 4.7 k Ω was chosen and the impedance magnitude deviation was calculated for a range of measured resistors.



Figure 3-6: Relative impedance magnitude error versus impedance magnitude for a fixed feedback resistor

As expected one can clearly see a vertical line around 150 Ω , caused by clipping of the opamp when applying this feedback resistor. These measurements were performed by either calibrating on a resistor of 630 Ω or 1.7 k Ω , thus dividing this impedance range further into two subranges. Beyond the 1 k Ω impedance point, the highest calibration value delivers most accurate results. Below 500 Ω the 630 Ω calibration resistor is more suited. In between 500 Ω and 1 k Ω no clear distinction can be made in deviation when calibrated on either of these

resistors, making this range the desired region to switch between these calibration values.

The division of each impedance range into two calibration subranges was done for each feedback resistor, thus giving a total of ten calibration resistors. Table 3-2 illustrates the resulting ranges, with Z_{min} and Z_{max} indicating the measurable impedance range for each calibration and feedback resistor. The feedback resistors were each chosen from the most common E12 series to lower design costs. Each switching point between two calibration resistors was experimentally determined by means similar to those shown in figure 3-6.

To increase ease-of-use for the end-user these fifteen resistors were incorporated in the device. Software controlled relay switching was applied to connect each feedback and calibration resistor when needed. Although these resistors could be switched by means of semiconducting components such as MOSFETS, parasitic elements, most dominantly capacitors, would hinder accurate impedance measurements. The use of relays avoids such problems, though putting some limits on the minimal size of the device.

Z min (Ω)	Z max (Ω)	R feedback (Ω)	R calibration (Ω)
10	68	250	39
68	125	250	97
125	1.2 k	3.8 k	630
1.2 k	2.2 k	3.8 k	1.7 k
2.2 k	13 k	56 k	7.4 k
13 k	28 k	56 k	19 k
28 k	216 k	820 k	120 k
216 k	410 k	820 k	313 k
410 k	2 M	10 M	1.5 M
2 M	5 M	10 M	3.8 M

Table 3-2 Impedance subranges for feedback and calibration resistors

3.3 System clock

The measurement frequency range at which impedance spectroscopy is performed on biosensors depends strongly on the type and layout of the sensor. Examples include a preferred excitation frequency of 80 Hz for polymer-based immunosensors [73], a kilohertz range for DNA mutation sensing [28, 101] and

up to 100 kHz for cell proliferation measurements [102]. Readout equipment specifically designed for a wide range of biosensors thus needs to support a wide spectrum of excitation frequencies.

In case of the AD5933 impedance analyzer IC the excitation frequency range is directly proportional to the applied clocking signal. This clock, being either the internal 16 MHz oscillator or an externally applied signal, is used to both generate the excitation sine wave and analyze the resulting measured signal. The excitation signal is provided by a 27-bit phase accumulated DDS core. The input of this phase accumulator is taken from user-settable dedicated registers. As shown in equation 3.2, since the absolute maximum applicable clock is equal to the internal clock of 16.6 MHz, users are given control over the excited signal up to a resolution of 0.119 Hz.

frequency resolution =
$$\frac{Clkmax}{\#bits} = \frac{16 MHz}{2^{27}} = 0.119 Hz$$
 (3.2)

The receive stage of the AD5933 chip performs a DFT on 1024 samples of the measured signal, thus transforming the signal from time to frequency domain. This enables signal analysis at the specified excitation frequency. The algorithm performed by this DFT is represented by equation 3.3, where x(n) is the output of the ADC, n is the sample number and X(f) is the signal amplitude at a specific frequency f.

$$X(f) = \sum_{n=0}^{1023} (x(n) (\cos(n) - jsin(n)))$$
(3.3)

When the internal oscillator of the AD5933 is used, the lower limit of the analyzable frequency range lies at 1/1024 of the 16.6 MHz oscillator frequency, being 16.3 kHz. As the excitation voltage upper frequency limit of the IC is set at 100 kHz, only a very narrow spectrum of frequencies is available to perform impedance measurements. Applying a range of external clock frequencies can broaden this spectrum. The required clock frequency range was determined by performing impedance sweeps in an excitation range from 10 Hz to 100 kHz with varying clock frequencies applied by a Keithley 3390 function generator. In figure 3-7 the deviation of the measured impedance magnitude is plotted in percent versus the measurement frequency for three different clock frequencies.

For each clock frequency a sweep was performed with 10 points per decade over the 4 decades. This figure not only illustrates that each clock has a distinct

usable range where the deviation lies well below 1 %, but it also shows that for each clock a single measurement frequency can be found were deviation is even significantly lower than 0.1 %. It should be noted that the point at which this effect occurs lies lower than the calculated minimum of 1/1024 of the clock frequency. This could indicate unreliable behavior when performing measurements around these frequencies.

Although it could be possible to sweep the entire range from 10 Hz to 100 kHz with a single clock frequency in the tenths of kilohertz range, it is not desirable to do so. The delay curve shown in figure 3-7 indicates that a low clock frequency of 62 kHz causes of measurement time as high as 18 s for a single sweep, while at 16 MHz clock frequency this time is only 7.6 s.



Figure 3-7: Relative impedance magnitude error and measurement time versus measurement frequency for three chosen clock frequencies

As an ideal situation every excitation frequency should have its distinct clock frequency, always keeping the measurement deviation around 0.1 % and ensuring a high measurement speed. Experimentally a fixed ratio of 1500 between clock and excitation signal was found. In order to achieve this fixed ratio, numerous solutions are available. Examples include dividing the frequency of a high-accuracy crystal oscillator, using a fully programmable microcontroller or dedicated ICs.

A Maxim DS1077 clock IC was chosen as external clock supply due to its small package size, ease-of-use and bus interfacing [103]. This I²C-bus programmable chip can deliver frequencies ranging from 8.1 kHz to 66.6 MHz at 8 Hz resolution by means of programmable dividers coupled to an internal oscillator. In figure 3-8 the measured magnitude deviation and master clock frequency (MClk) versus excitation frequency are shown utilizing this IC with a programmed clock/excitation ratio of 1500. This measurement was performed on an 820 Ω resistor, thus central within the impedance range corresponding to the second feedback resistor, as documented in table 3-2. The deviation in measured magnitude was calculated in respect to the actual resistor value measured with a Keithley 2000 multimeter and expressed in percent.



Figure 3-8: Relative impedance magnitude error and clock frequency versus measurement frequency

Since the DS1077 IC is limited in upper frequency to 66MHz, the fixed clock/excitation ratio of 1500 can only be applied for excitation frequencies up to 44 kHz. This can be seen by clock frequency clipping in figure 3-8. The lowest measurement frequency is limited to 5.4 Hz when using the fixed clock/excitation ratio. It is however possible to perform impedance spectroscopy over 4 decades, i.e. from 10 Hz to 100 kHz while keeping measurement deviation around 0.1 %. The measurement time of these sweeps, performed with 10 points per decade over 4 decades, was 11.2 s. This makes the DS1077

IC a viable clocking solution for the AD5933 and allows for fast, reliable, broadspectrum frequency sweeping.

In respect to frequency sweeping it should also be noted that the AD5933 impedance chip on itself is only able to perform linear frequency sweeps. Since the available excitation range was increased to 4 decades, logarithmic sweeping is preferred. This was achieved by forcing the IC to perform sweeps of a single frequency. By logarithmically dividing the starting frequencies of each single-frequency "sweep", a logarithmic sweep is simulated. The AD5933 is not able to perform linear sweeps without communicating with the I²C master for each point in the sweep, since no memory for data storage is present in the IC. The resulting computing overhead and measurement time increase in using this method is thus minimal.

3.4 User control and measurement routine

The AD5933 impedance chip is not able to perform measurements by itself nor does it have on-board memory to store data. All interfacing to the chip is done by an I²C bus. This bus topology utilizes an open-drain clock (SCL) and data (SDA) line and operates at speeds ranging from 100 kbit/s up to 3.4 Mbit/s. The AD5933 supports a speed of 400 kbit/s and functions as a slave to a single master, which can be a processor controlling the bus. Also the DS1077 clock chip, discussed in the second paragraph of this chapter, can be controlled by I²C protocol. To ease design all other ICs included in the system were chosen to run on this bus. A Texas Instruments PCA9536 I²C I/O expander is for example used to control the channel switching relays.

Since the BioZ^o device will be completely controlled by LabVIEW software running on a PC, a way of communicating between the PC and the I²C bus is required. Standard USB protocol, present on any PC, was chosen as communications method. Interfacing USB protocol to the I²C bus was done by a Future Technology Devices International Ltd. (FTDI) FT232R chip [104]. This USB-to-Universal Asynchronous Receiver/Transmitter (UART) converter provides a fully integrated way to convert USB signals to asynchronous serial data. The serial line signal can be interpreted by a microprocessor acting as an I²C bus master. A Microchip Technology Inc. 16F627 PIC microcontroller was chosen to serve this purpose [105]. It should be noted that this simple, low-cost processor does not have any other purpose in the device than acting as the UART to I²C converter and buffer since all software runs on a PC. The BioZ^o device is recognized by a connected PC as a virtual COM-port, which eases software development.

Depending on the field of application, a universally usable device for biosensor readout should have numerous ways of communicating data with the end-users. Since the BioZ° device is controlled completely by a PC, a software interface can be written to satisfy the needs of a certain user group. Running the unit under LabVIEW, a graphically programming environment, facilitates this process. Figure 3-9 illustrates the measurement routine that was implemented in the software. Upon startup an array of frequencies is generated from spectrum parameters set by the user. The first of maximum eight measurement channels is switched on and the DS1077 master clock frequency and AD5933 measurement frequency are set from the first value in the frequency array. After a start command is given to the AD5933 the program waits until values are present in the DFT registers. If these values are not correct, i.e. not within range of the current feedback resistor in the current-voltage converter OPAMP, this resistor is changed to another value as discussed in paragraph 3.3. After each measurement the selected frequency is incremented from the frequency array and the routine is repeated until the sweep is complete. After turning the AD5933 in power down mode the measurement is repeated, if needed on a different channel.



Figure 3-9: BioZ° measurement routine

3.5 Power supply and PCB layout

When dealing with miniature, stand-alone measurement equipment special care should be taken towards power supply design. The BioZ° device, which is powered by USB, operates on 5 V DC voltage. Since the OPAMPs inside this unit require a positive and negative voltage, a Recom regulated DC-DC convertor was used [99]. This convertor not only delivers +5 V and -5 V, but also enables splitting between a digital supply (the USB voltage) and an analog supply (+/5 V). One downside of this mechanism is the introduction of significant noise [106]. Both the digital and analog supply are brought down to 3 V by Analog Devices ADR433 regulators to supply the AD5933 impedance chip. All other ICs are powered by the digital 5 V supply. Components in the impedance signal path are supplied with the analog +/-5 V. The device is rated at a power consumption of 100 mA, with the relays for channel and feedback resistor switching as the major consumers. Each relay consumes 21.1 mA. At any given time two relays are activated, one for a channel and one for a feedback resistor, thus the total relay power consumption lies at 42.2 mA. In worst case scenario, when a USB 1.0 port is used with one unit load available, maximum USB power supplied lies at 100 mA, thus sufficient for the BioZ^o device [107].

Since all components are directly or indirectly hooked up to the same supply voltage, noise can circulate between components. One common source of digital noise is caused by current spikes due to clocking inside digital ICs or inside dedicated clocking chips or voltage regulators. Also any component that switches between voltages levels, e.g. for switching the on-board channel relays, causes current spikes on the supply rails. Unwanted voltages can occur if these currents are flowing through path with non-zero impedances.

Instead of working at a fixed clocking frequency, as indicated in paragraph 3.3, the frequency put out by the DS1077 IC varies from 15 kHz to 66 MHz. Furthermore, the voltage converter used to convert the 5 V supply voltage into +/-5 V has a switching frequency of 200 kHz. When dealing with these high frequency noise sources, specific signal decoupling is essential. The most common way to dismiss of this noise is by means of decoupling capacitors. In its simplest approach a decoupling capacitor behaves as a frequency dependent shortage between supply voltage and ground.

$$Z_{ideal} = \frac{1}{2\pi fC}$$
 and $Z_{practical} = ESR + ESL. 2\pi fC + \frac{1}{2\pi fC}$ (3.4)

As indicated by equation 3.4, the impedance of a capacitor is reversely proportional to the frequency of the applied signal. The value of the capacitor can thus be chosen to result in an as low as possible impedance for the unwanted frequencies. In practice however а capacitor has series inductance (ESL) and resistance (ESR) in the form of leads and traces in series with the capacitance. This causes the resulting impedance to be low only at a certain resonance frequency [108]. It is therefore common to use a set of capacitors with different values and different materials in parallel to decouple a certain frequency range [109]. Both an electrolytic 10 µF and a ceramic 100 nF capacitor were placed at the power supply trace of each IC. The DS1077 clock chip was decoupled with an extra ceramic 10 nF capacitor to remove high frequency interference. To avoid interference of any external noise sources and to avoid limit inductance these capacitors were chosen in Surface Mount Device (SMD) 0603 packages and placed as closely as possible to the power connection pins of each chip. Increasing distance between decoupling capacitors and supply pins causes an increasing parasitic inductance in series with the capacitance. This way an LC resonating circuit is created, not only opposing the decoupling effect but also causing oscillation on the power supply voltages.

Aside from carefully chosen decoupling capacitors, placement of the components and power planes plays an important role in noise reduction. Since it was possible to route all signal paths on two layers of a printed circuit board (PCB), a four layer PCB incorporating a power and ground plane as inner layers was chosen. This setup has some advantages. Capacitive coupling between the power and ground plane is increased, providing better power supply filtering and resulting in a lower number of necessary decoupling capacitors [110]. Also, since the choice was made to use a ground plane within the whole area of the PCB, grounding loops are removed and ground impedance is significantly decreased [111]. This allows for better signal conducting towards the power supply ground, in this case the USB port, which decreases grounding noise. The layout of the BioZ° device power plane is shown in figure 3-10. It should be noted that the ground plane has a similar layout.



Figure 3-10: BioZ° power plane layout

One can clearly distinguish three different subplanes in this power plane. It is a common technique in mixed signal design to split the PCB in an analog and digital part, each with its own power planes. These planes can be brought together only at the power supply to minimalize interference between the different subcircuits. When conversions occurs between analog and digital signals it is however common practice to create a certain carefully designed shape underneath the analog-digital (AD) or digital-analog (DA) convertor, where both planes are brought together [110, 111]. This path provides however a bottle neck for the ground signals. It is therefore common in these layouts to place noisy, high-speed digital components closer to the power connectors than low-speed analog circuitry. This prevents any digital noise from passing underneath the analog planes and specifically reduces noise caused in the analog-to-digital ground path.

Since the AD5933 impedance chip is the only component which operates with both analog and digital signals, connection between the analog and digital ground plane is made directly underneath this IC. The relays used to switch between measurement channels and feedback resistor in the signal path OPAMP are controlled by digital signals. Inside these relays there is however a galvanic separation of digital control signal and analog measurement signals. Therefore the relays were placed exactly on the separation between analog and digital planes. The analog power plane was further divided into a +5 V and -5 V plane. It should be noted that a similar, though slightly more complex layout was also used on the AD5933 evaluation board discussed in the first paragraph [90].

A mass-produced Acrylonitrile-Butadiene-Styrene (ABS) plastic enclosure by Hammond Manufacturing was chosen to house the circuitry. The plastic compound selected does not provide additional electro-magnetic (EM) shielding for the PCB. This choice is based upon the fact that biosensor platforms that connect to the unit very seldom provide EM shielding and would therefore eliminate any shielding effects present in the enclosure.

3.6 System evaluation

The BioZ° device is designed to operate within an impedance range of 10 Ω to 1 M Ω , enabling the readout of a large variety of biosensors. The accuracy of the unit is evaluated by measuring a set of resistors within this range. The IEC 60063 E12 resistor range was chosen for this purpose due to its high availability and almost logarithmic scale. Each decade is divided into 12 subdivisions according to the Renard standard series. 1% metal film resistors were used for the almost flat frequency response within the measured spectrum of 10 Hz to 100 kHz and high temperature tolerance. The impedance magnitude should equal the resistance, while the phase should approach zero for the entire frequency range. Figure 3-11 shows the deviation in impedance (in percent) and phase (in degrees) with respect to the measured set of resistors for the BioZ° unit. These measurements were performed at a fixed frequency of 1 kHz, thus central within the logarithmic frequency spectrum of 10 Hz to 100 kHz.

The deviation in impedance lies well below 1 % for the largest part of the resistor range. The average magnitude deviation lies at 0.44 %. Furthermore, the deviation in phase is always less than 1°. A significantly lower deviation can be seen around resistor values approaching the on-board calibration resistors, shown in table 3-2. Incorporation of a lager set of resistors would decrease deviation but would also require much more computational power, thus reducing measurement speed. It would however be possible in particular applications to build calibration resistors approaching the value of a specific sensor type.

Another important aspect when evaluation the performance of devices for biosensor readout is long-term stability. High accuracy should not only be achieved momentarily when connecting a sample, but should be maintained for the duration of the measurements. This duration can vary from minutes, e.g. for synthetic MIP sensors, to several days in cell proliferation measurements. In general two problems can arise during measurements, commonly referred to as noise and drift. Electronic noise exists in all circuits and can be seen as random fluctuation around a mean value. Drift expresses relatively long-term unidirectional value changes.



Figure 3-11: Relative impedance magnitude error and phase error versus impedance magnitude

Furthermore, although measurements on resistors give a good indication of device accuracy, these components cause no phase shift between voltage and current, nor do they inhibit charging behavior and resulting time constants. To analyze noise and drift parameters of the BioZ^o units on more complex circuitry, an equivalent circuit of a measurement cell used for DNA-denaturation measurements was constructed. A resistor of 84 k Ω was placed in parallel with a 3 nF capacitor to emulate the interfacial double layer of a diamond substrate placed in conducting PBS solution [112]. A 306 Ω resistor was placed in series with this circuit to take into account liquid resistivity and losses in connectors and cables. Figure 3-12 illustrates this equivalent circuit.

The circuit was continuously measured for a duration of 24 hours with a measurement time set at 44 s per sweep, resulting in a total of almost 2000 sweeps. The difference between the measured and average magnitude value was calculated at the beginning, the center and the end of the frequency spectrum. Figure 3-13 shows this relative magnitude for a frequency of 15 Hz, 1 kHz and 100 kHz. No distinct drift is present in the signal as the measured magnitude fluctuates around its average value for the complete 24 hours duration. In general the noise level lies below 0.4 %.



Figure 3-12: Equivalent circuit of a biosensor measurement cell



Figure 3-13: Relative impedance magnitude drift of an equivalent RRC circuit versus time

The noise level is further analyzed by performing impedance measurements on a nano-crystalline diamond (NCD) sample, commonly used at as platform for DNA sensors [113], mounted in a liquid cell. The cell was sealed and kept at a stable temperature of 37 °C to rule out impedance changes due to temperature fluctuations. Measurements where performed on a frequency of 1 kHz, thus central within the available frequency spectrum. In figure 3-14 the magnitude and phase are plotted in respect to their average values over a time of one hour. The magnitude noise level is within 0.25 % while the maximum phase deviation lies at 0.2 °.

When looking at the impedimetric response of biosensors to target molecule detection, typical values can be seen in the range of several percent, even for detection of concentrations just above the detection limit of the sensor itself [28, 73]. Therefore the device accuracy and stability are well within specifications for accurate biosensor readout.



Figure 3-14: Relative impedance magnitude and phase noise versus time for a diamond substrate at a measurement frequency of 1 kHz

3.7 Biosensor readout

3.7.1 Temperature controlled measurement setup

Before performing actual biosensor measurements device performance was verified on the single-channel measurement cell discussed in paragraph 2.2. A $10 \times 10 \text{ mm}$ boron-doped diamond substrate, a common immobilization platform for DNA sensors, was mounted into the cell, after which the cell was filled with PBS solution. A stepwise temperature profile was applied, starting

from 35 °C and increasing up to 90 °C in steps of 10 °C, after which the cell was allowed to cool down again in steps of 10 °C by means of convection. The thermocouple mounted in the copper lid, thus directly under the diamond substrate, was used as a reference for the PID control. Liquid temperature is monitored using a second thermocouple.



Figure 3-15: Temperature profile applied on a liquid cell setup containing a boron-doped diamond sample in PBS and the effect on the measured impedance versus time

The diamond-PBS interfacial capacitance was most dominant around 12 kHz. Impedance was continuously monitored at this frequency and is plotted together with both copper and liquid temperature in figure 3-15. As expected the measured impedance is inversely proportional to the applied temperature [114]. To further investigate this effect, the Nyquist plots of the complex impedance at different temperature level is plotted in figure 3-16. An increase in temperature causes a decrease in both real and imaginary impedance. Changes in real impedance are related to resistive components, in this case most likely related to the purely resistive liquid. Higher temperature causes higher ion mobility in liquids, thus increasing conductivity and decreasing impedance. The imaginary impedance can relate to the diamond-PBS interfacial capacitance. Boron-doped diamond behaves like a semi-conductor, where an increase in temperature increases the number of free electrons and thus also increases conductivity.



Figure 3-16: Nyquist plots showing imaginary versus real impedance at different temperature levels for boron-doped diamond in PBS

3.7.2 Synthetic MIP-based sensors

As a next step in device performance verification a MIP nicotine sensor was measured upon addition of varying concentrations of target molecule. PPV-backplane MIP and a NIP samples were mounted into a four channel wet cell similar to the setup discussed in paragraph 2.1 with temperature at the copper substrates set at 37 °C.

Concentration curves are commonly used to demonstrate the applicability of biosensors in clinical settings. The impedimetric response of the sensor is measured when exposed to a range of (clinically relevant) concentrations. The samples were allowed to stabilize for about 1 hour after which increasing concentrations of nicotine were added, resulting in a total concentration range of 100 nM to 2 μ M. The concentration curve in figure 3-17 shows the impedance magnitude response of the differential MIP-NIP signal in respect to the values measured just before addition of the first concentration. These measurements were performed at a frequency of 150 Hz. A differential response of 0.1 % is measured at the lowest detectable concentration of 200 nM, while the sensor seems to saturate at concentrations slightly above 1 μ M.



Figure 3-17: Concentration curve displaying relative impedance magnitude change versus concentration for a MIP-based nicotine sensor

These measurements were repeated on a MIP-based histamine sensor and the effect of binding events in the sensor layer on the measured impedance was analyzed. Figure 3-18 shows the response of the differential MIP-NIP impedance magnitude upon addition of 2 nM of histamine. Upon addition this target molecule a slight decrease in magnitude can be seen, most likely due to sudden movement in the liquid, after which the magnitude of the MIP impedance increases significantly in respect to the impedance of the NIP. A much higher response occurs in respect to figure 3-17, which could be related to differences in the sensor layer due to the different target molecule or the fact that sample are manually prepared and mounted into the measurement cells.



Figure 3-18: Relative differential MIP-NIP signal response versus time upon histamine addition

To gain insight into the mechanics of the impedimetric MIP readout the real and imaginary parts of the impedance were plotted. Figure 3-19 (top) shows the real part of the MIP impedance at the selected frequency of 150 Hz versus time, while the imaginary part can be seen in figure 3-19 (bottom). Upon addition of histamine the real part of both the MIP and NIP impedance increases towards a stable level, which is most likely due to changes in the medium upon histamine addition. The difference in slope is possibly a consequence of the stabilization period of the MIP. Addition of histamine causes a decrease in imaginary MIP impedance of about 20 % while the NIP decreases only 2 %.

It can thus be concluded that signal response in the MIP particles can be most dominantly seen in the imaginary impedance, which is related to capacitive components. This is possibly due to the cavities inside the MIPs, which become filled with the negatively charged histamine molecules instead of the neutrally charged, highly conducting PBS. This causes an increase in capacitance, resulting in a decrease in capacitive reactance and thus also a decrease in imaginary impedance.



Figure 3-19: Real (top) and imaginary (bottom) impedance of the PPVimmobilized MIP and NIP samples in respect to their initial values versus measurement time

3.7.3 Biological cell proliferation

The previous paragraphs described measurements performed on a temperature controlled biosensor measurement cell and biomimetic MIP sensor. As a final step in device verification biological cell proliferation was monitored. An amount of 30000 of HEK, BV2 and CHO cells was simultaneously seeded into different wells of the 96-channel multiplexer described in paragraph 2-2. Figure 3-20 shows the initial Bode plots for these wells, where a clear distinction between the three cell types can be seen in the high-frequency part of these plots. At these high frequencies the impedance is mostly due to resistive components, as the phase approaches zero. This resistivity is directly related to surface coverage of the cells on the electrodes, which is possibly influenced by the diameter of the cells [115]. The 15 μ m diameter CHO cells show a significantly higher resistance than the 10 μ m HEK cells, which have in turn a much higher resistive component than the 8 μ m BV2 cells.



Figure 3-20: Bode plots showing impedance magnitude and phase versus frequency for three types of cells commonly used in biomedical research

To illustrate the possibilities of this setup for proliferation monitoring, an amount of 1000, 3000, 6000, 10000 and 30000 BV2 cells was seeded, after which impedance was continuously monitored in the highest available decade (10 kHz to 100 kHz). Figure 3-21 shows the impedance changes for each of
these initial amounts in respect to the lapsed time. Since the measured impedance is related to the amount of cells, one can clearly see the evolution of population size for each concentration of seeded cells.



Figure 3-21: Impedance magnitude change of different initial population sizes of BV2 cells versus time

A drop in impedance is noticed on all wells, most likely due to nesting of the cells on the electrodes. After this stabilization, an increasing impedance is observed on the wells containing the relatively high amount of 30000 cells. At this point the population started its exponential growth. The wells containing lower amounts of cells start this doubling period after longer time intervals. This growth effect could not be detected for the lowest population of 1000 cells. The impedance reaches a distinct maximum after a certain time interval, probably when maximum population size is reached. After this maximum is reached, impedance decreases almost exponential, most likely due to cell death.

3.8 Result and discussion

A miniature impedance spectroscopy device, especially aimed at biosensor readout in lab settings, was presented in this chapter. The Analog Devices AD5933 impedance analyzer IC was chosen as a basis for device design. As was shown in paragraph 3.2, the excitation signal put out by this chip is attenuated and DC bias voltage is removed to avoid any interference with biological samples. The resulting voltage is amplified by an OPAMP with a set of relayswitchable feedback resistors to enable sensing of impedance range from 10 Ω to 1 M Ω . This way, the complete impedance range is divided into 5 sub ranges, each with 12-bit measurement resolution. Paragraph 2.3 illustrated the steps taken to measure this impedance in a frequency spectrum from 10 Hz up to 100 kHz. Both the impedance range as the frequency spectrum were carefully chosen to read out a wide variety of biosensors. The complete circuit diagrams and bill of materials can be found in appendix 4.3.



Figure 3-22: BioZ° impedance spectroscopy unit

The completed BioZ^o unit is shown together with a 3D computer aided design (CAD) drawing in figure 3-22 with a one euro coin for size reference. Connection can be made to the 8 measurement channels by means of subminiature SMB connectors on top of the device while a USB and custom peripheral port are present for connecting respectively a controlling PC and peripheral equipment.

Device performance was first verified by measurements on passive components. An average measurement error over the complete impedance range of 0.44 % was achieved while no distinct drift could be seen when performing measurements over long period of time.

Next the device was connected to a biosensor measurement cell after which this cell was exposed to a temperature profile. Temperature-induced effects on the impedance, most dominantly caused by double layer capacitance and liquid resistivity changes, were measured. Impedimetric response of a synthetic MIP sensor was subsequently characterized, resulting in concentration curves. It was noticed that this response was most dominant in the imaginary impedance. Last but not least biological cell proliferation was monitored.

The measurements indicate the possibilities of the presented device in sensor setup characterization, biomimetic sensor readout over a range of concentrations and continuous biological cell proliferation monitoring over a period of several days. This would make the PC-controlled unit suited for application in biosensor labs.

Chapter 4

A standalone point-of-care impedimetric biosensor readout system

"BioZLCD"

In recent years an increasing interest has risen towards point-of-care devices for rapid onsite diagnostics and reduced diagnosis time. Since biosensors allow for quantization of biomedically relevant molecules research is constantly being conducted towards increasing the applicability of these sensors in such settings. This chapter describes the development of a readout device, named BioZLCD, specifically targeting point-of-care applications. The name originates from the combination of impedance based biosensor readout (BioZ°) and convenient user interfacing by means of an LCD touchscreen. Measurement circuitry is kept identical to the circuit present in the BioZ° device and essential design aspects for user stand-alone operation are discussed in detail in this chapter.



Figure 4-1: BioZLCD block diagram

A stand-alone measurement system requires, aside from an internal processor, data storage and power supply, some way of communicating data to operators. Several communication protocols were implemented in the BioZLCD device to enable both wireless and Ethernet-based control as well as data access. Furthermore the user has complete control over the measurements by means of a touchscreen. This way a bridge is built between technology commonly present in present-day smartphones and specialized measurement equipment and a basis is set for commercially available, impedance based biosensor devices. The block diagram in figure 4-1 shows the basic elements present in the system.

4.1 Embedded processing

Contrary to the previously described BioZ° unit, the BioZLCD device requires all software to run and memory to be present on board the device. In order to do so, a relatively powerful programmable processor core needs to be implemented inside the system. In contrast to a separate processor, memory and peripherals, as present in most common-days computers, an embedded microcontroller (MCU) is more suited for small, portable applications. Microcontrollers typically integrate many of these essential components into a complete system-on-a-chip (SOC). When looking at the guite large microcontroller market, a distinction is often made based on the instruction and data width. This way, these SOCs can be divided into 8, 16 or 32 bit processors. Since the impedance data is coded into 12 bit, one would expect a 16 bit processor to be sufficient to control, manipulate and store the measurements. However, manipulation of decimal numbers is required in impedance calculation. This can be a tedious task for many processors, hence why a separate floating-point core is present in some embedded systems. Furthermore a floating-point number consists of a separate significant digits and exponent bits, placed in the same data block, causing the need for larger data widths. A 32 bit processor thus seemed most suited for the application.

When looking at the 32 bit embedded processor market, the most dominant technology is Acorn RISC Microprocessor (ARM) based. Not a semiconductor manufacturer itself, ARM supplies intellectual property and licenses third party manufacturers to develop and produce ARM chips. This technology can be found in most hand-held embedded devices, including smartphones and tablet PCs. Figure 4-2 shows the different types of available ARM cores upon writing of this work [116].



Figure 4-2: ARM processor product range

A lot of the cores have proven themselves extensively in numerous applications. ARM7 technology is for example used in the Apple iPod MP3 player and a large range of Nokia cell phones. The Cortex-A8 core on the other hand is implemented in the Apple A4 and Samsung Hummingbird SOC, present in respectively the iPad tablet PC and iPhone 4 smartphone and the Galaxy range of smartphones [117, 118]. ARM technology has also been previously used in the development of portable biosensor systems [119].

When looking at the target application it becomes clear that the chosen microcontroller will have to support several bus protocols in order to communicate with all the peripherals on the device. The memory demand for this application is an estimated 300 kB of Flash and 36 kB of RAM. Aside from the mentioned bus support, these relatively demanding characteristics are mainly based on the need for graphical processing to display the measurements in an appealing way. In order to process the floating point data there are two possible options. A microcontroller can be selected which has a hardware floating-point arithmetic. The most common floating-point unit (FPU) is the Vector Floating Point (VFP) architecture. The second option is to select a 32-bit microcontroller and run a so called soft-float to handle these floating point operations in a software emulator. Receiving new impedance data over the I²C bus takes much longer than the amount of clock cycles needed to handle the previously obtained data. Therefore the second, software-emulated option was chosen to handle the floating point data.

The Cortex-M3 core was chosen as processor architecture for the BioZLCD device. This range of microcontrollers, based on ARMv7-M architecture, is fine-

tuned for cost-efficient, low-power applications. Upon writing the M3 processor was also the most widely available and supported processor within this range. It should be noted that some of the processors mentioned in figure 2-9 were not yet in production when developing the device. The choice was made to implement the STM32F103VET6 manufactured by ST Microelectronics. This Cortex-M3 based MCU has 512 kB of FLASH on-board combined with 64 kB of RAM and runs on a 72 MHz clock [120]. Several communication interfaces are present, including 2 I²C and 3 Serial Peripheral Interface (SPI) busses as well as a direct USB 2.0 interface. This MCU is available in numerous memory density packages allowing the user to switch to a lower or higher memory density package whenever needed. This is a great advantage for prototype to end-product designs because there is no need to create a new hardware layout or change any application code. All the devices are pin/peripheral compatible.

One disadvantage of microcontrollers in respect to other technologies, for example Field Programmable Gate Arrays (FPGA), is the linear instruction execution. When not taking into account interrupts, instructions are executed in a fixed order within an endless loop. The BioZLCD device needs to perform different tasks quasi-simultaneously. If an impedance measurement is running, user control should still be enabled. Furthermore it is desirable to constantly update measurement data via Ethernet network or Bluetooth. This requires several processes to run simultaneously. In order to do so a Real Time Operating System (RTOS) is required. Software failure is an important cause for medical device faults and recalls [121]. An RTOS is especially designed to run applications with very precise timing and a high degree of reliability, reducing downtime and faulty measurements. A number of third party vendors offer RTOS for ST Microelectronics MCUs. Many of these provide a full range of features that can be used on the developed BioZLCD device. A Micrium μ C/OS-II real-time kernel, specifically designed for the medical market, was implemented [122]. This RTOS can run up to 64 tasks and requires about 18 kbytes of memory. It is worth noting that for university educational purposes and research the RTOS does not require licensing.

4.2 User interfacing and measurement routine

A tendency in devices currently being released or under development for (bio)medical applications is the incorporation of touch-screen displays. It has been shown that patients can easily adapt to touch screen technology for fast diagnosis systems [123]. Examples of such applications can be found in quality of life control for terminal patients [124, 125]. Therefore a touchscreen was chosen as a convenient way of user control, e.g. setting up measurement parameters and monitoring results, in the BioZLCD device. The upcoming

market of touchscreen devices, dominated by smartphones and tablet PCs, has caused an increase in technology development and drop in implementation cost [126].

Although the STM32 is not equipped with an on-chip liquid crystal display (LCD) controller, this microcontroller has an embedded flexible static memory controller (FSMC) instead, which can be used together with the on-chip Direct Memory Access (DMA) controller to implement a direct drive for thin film transistor (TFT) LCDs, as illustrated in figure 4-3. The LCD module interfaced with the FSMC is a Quarter VGA (QVGA) TFT LCD module based on the ILI9320 driver by ILITEK. The Analog Devices AD7843 is used on this module as a four-wire resistive touchscreen controller with a 12-bit successive-approximation ADC for accurate, single-point touch detection. This chip communicates with the central microcontroller over an SPI bus. The touchscreen with 320 x 240 pixels provides the users with full control over measurement settings and allows for results analysis in a graphical manner.



Figure 4-3: LCD and microcontroller interfacing

Common day commercial biosensor applications could benefit from TCP/IP protocol implementation, enabling remote monitoring over a standard local area network (LAN) or even over the Internet [127, 128]. Aside from being a convenient feature in lab environments, consumer healthcare could be improved by remote monitoring. This would mean a significant increase in patient security and comfort, while reducing diagnosis time [129]. An Ethernet communication circuit was implemented in the BioZLCD device to enable this network-based control. At the core of the sub-circuit is the Microchip ENC28J60 Ethernet Controller [130]. This controller communicates with the ARM Cortex M3 microcontroller, running the webserver and generating a W3 compliant HTML website the user can visit via the local network. The interfacing with the microcontroller is achieved via a SPI bus.

Users can log in onto a user-defined password-secured website where, depending on pre-set user privileges, measurements can be monitored or control can be taken over the device. Remote recalibration and configuration of measurement settings is possible as well. User Datagram Protocol (UDP) is selected as a suited protocol to achieve this communication between the BioZLCD device and a host. UDP allows computer applications to send messages (datagrams) to other hosts on an Internet Protocol (IP) network without requiring prior set up, thus reducing complexity and network load.

By implementing both a touch screen and an Ethernet module, direct device control and long-distance access is covered. It is however desirable for certain applications to wirelessly monitor the measurements over a short distance. Bluetooth was chosen as a protocol to serve this purpose. Aside from being used in wireless patient monitoring systems [131, 132], this protocol was for example also used for crowd movement studies [133]. It should be noted that Bluetooth is a patented technology, meaning that a free embedded Bluetooth stack is non-existent and the use is only licensed to a patent qualified device. The BT-23 module developed by Amped'RF is a complete Bluetooth licensed radio solution [134]. This module was interfaced to the central microcontroller by UART. Bluetooth protocols up to v2.1 at typical speeds of 1.5 Mbit/s are supported. The module support Serial Port Profile (SPP) stack, allowing users to communicate with the chip trough a virtual COM-port. This simplifies implementation of Bluetooth protocol in the microcontroller.

Aside from these communication protocols with which data is transferred, the measurements are also stored on an on-board Secure Digital (SD) card. Hardware design is simplified since the MCU supports direct connection to an SD card. Due to design constraints it is however not possible to remove this card from the device. Doing so would require the enclosure of the device to be either very thin in order for the card to be reachable, or the card socket would be placed on the very edge of the PCB, resulting in mechanical weakness. The user can instead plug the complete BioZLCD unit into an USB-port, after which data stored on the SD card can be accessed. This card also functions as a backup for sudden connection loss when using the different communication protocols.

Finally two custom interface ports were placed on the unit for connection to external equipment. It is for example common to interface impedance analyzers to multiplexers in order to read complete arrays of biosensors. The interface ports supply the I^2C bus protocol in order to establish communication to other I^2C bus controlled devices, for example the custom multiplexer used for cell proliferation measurements.



Figure 4-4: RTOS tasks and measurement routine

A number of tasks were implemented in the RTOS to handle the different user interface features. Figure 4-4 illustrates these tasks together with the measurement routine, being a modified version from the routine discussed in paragraph 3.4. Upon startup of the software in the main() function a task called APP_START is created in the RTOS. This task will perform hardware startup routines, e.g. resetting the Bluetooth module, as well as calling the OSStart method to allow the RTOS to start multitasking. At this point the processor can switch between six different tasks, each with a set priority. User interface events are given the highest priority to prevent the system from unresponsive to user interaction. This includes graphic display on the LCD and handling touchscreen events. Low priority tasks like the continuous blinking of an LED indicator are non critical to either user interaction or performing measurements. The measurement routine can either be started from touch events, from the website or via Bluetooth commands. In either case a command is given to the UC_GUI task which will run the routine and display measurements.

4.3 Power supply and PCB layout

As the measurement circuitry is identical to the BioZ^o device, power supply and PCB layout for this sub circuit was kept similar in the BioZLCD unit. A separation is made between digital +5 V and +3 V voltages, used for ICs and control logic, and +/-5 V analog voltages. Connection between the digital and analog power and ground planes is again made underneath the AD5933 impedance analyzer chip. The BioZLCD device has an extra sub circuit, consisting of the ARM microcontroller with communication peripherals. This control logic operates completely on digital voltages, so no analog-digital splitting was required. Careful layout was however necessary to avoid interference between this digital sub circuit and the analog/digital measurement circuitry. Since the I²C bus is the only means of communication between the 2 sub circuits, a component layout was chosen as shown in figure 4-5. The left part of the PCB contains the ARM microcontroller together with the peripheral ICs, including the Ethernet and Bluetooth controller. The right side contains the impedance measurement circuitry with digital control signals, further divided into the analog and digital part similar to the BioZ° device. This layout helps to further reduce the impact of any noise generated inside the digital circuitry based around the central microcontroller on the impedance measurement subcircuit.



Figure 4-5: BioZLCD PCB layout

An internal rechargeable 950 mAh Lithium-ion (Li-ion) battery supplies power to the BioZLCD unit. The main advantages of this battery over other types, e.g. nickel-metal-hydride, are its high voltage and energy density and absence of memory effects. Cost is however significantly higher and specialized charging circuitry is needed. A Linear Technology LTC4045 standalone linear Li-ion battery charger with temperature compensation was used to convert the 5 V voltage of a connected USB-port to the desired recharge voltage and current. Power consumption of the microcontroller and peripheral circuitry is rated at 130 mA. As in the BioZ^o unit, the impedance measurement circuitry consumes 100 mA, thus resulting in a total battery life of about 4 hours.

The circuitry was placed inside an ABS plastic enclosure produced by Hammond Manufacturing. As is the case with the BioZ^o device, no extra EM shielding is present in the device.

4.4 System evaluation

Although measurement circuitry in the BioZLCD system was kept identical to the BioZ° unit, the addition of complex digital circuitry could cause interference with the analog measurement circuitry and hinder accurate readout. Accuracy measurements on the E12 set of resistors, as done in paragraph 3.6, were repeated. Figure 4-6 shows the deviation in magnitude, in percent, and the deviation in phase, in degrees, in respect to the measured resistor.



Figure 4-6: Relative impedance magnitude error and phase error versus impedance magnitude

The BioZLCD device achieves a slightly higher accuracy the BioZ° with an average impedance magnitude error of 0.22 %. The maximum error is well below 1 % in magnitude and 1 ° in phase. Table 4-1 gives a comparison between the performance of the BioZ° and BioZLCD devices. The average error expresses the average measurement error over the impedance magnitude range of 10 Ω to 1 M Ω . The standard deviation indicates the dispersion from this average; a low value indicates that the different calculated deviations lie very close to the mean. A worst-case scenario value can be seen in the maximum deviation, while the 95th percentile stands for the maximum error of the 95 % most accurately measured resistors. All values are expressed in percent. The measurement circuitry implemented in both devices is similar and care has been taken to avoid interference on board design level. The difference in accuracy is thus most likely due to interference coming from digital circuitry and power supply. Since the BioZ^o unit is powered via USB-bus, a high percentage of the power supply noise is caused by noise coming from the connected PC. Power supply noise is filtered extensively by means of decoupling capacitors and power supply splitting, yet a significantly higher accuracy is achieved with the batterypowered BioZLCD unit. Both devices achieve however high enough accuracy for biosensor readout.

	BioZ°	BioZLCD
Average error	0.40	0.22
Standard deviation	0.71	0.18
95 th percentile	0.95	0.59
Maximum deviation	4.47	0.84

Table 4-1 BioZ° and BioZLCD performance, all values in %

As the BioZLCD is intended for point-of-care applications and an on-board processor allows for complex computations, software algorithms could be implemented to calculate essential measurement parameters. This way, the ideal measurement frequency for a specific type of sensor can be determined from Bode or Nyquist plots. Measurements on the equivalent biosensor circuit shown in figure 3-10 were compared with a high-end commercially available impedance spectroscopy unit to verify the abilities of the device for correct measurement and display of frequency-dependent magnitudes and phases. An HP4194A gain/phase analyzer was used to serve this purpose. This device was released in the early 1990s and has proven itself on numerous types of biosensors [40, 51, 72, 135]. It should be noted that the average impedance accuracy of this device is 0.17 %, thus only slightly better than the BioZLCD unit [136]. It is therefore not possible to use this device as a reference unit for construction of accuracy graphs similar to figure 3-1 and 3-2. Furthermore, the maximum frequency spectrum is 100 Hz to 40 MHz, thus rendering comparison in the lowest decade of the developed units, i.e. 10 Hz to 100 Hz, impossible.

Figure 4-7 gives a comparison between the Bode plots of the discussed equivalent circuit, when measured on the HP4194 and the developed BioZLCD unit. One can see similar results on both analyzers. The deviation between the magnitude plots at 10 kHz corresponds to the point where a change is made in feedback resistor in the measurement circuitry. The device detects a magnitude value out of range for the current feedback resistor and thus switches resistors at the next measured frequency point. This causes a brief increase in deviation.



Figure 4-7: Equivalent circuit Bode plots, expressing impedance magnitude and phase versus frequency, as measured on the HP4194 and the BioZLCD unit

4.5 Biosensor readout

MIP sensors and reference NIPs were used to verify performance of the device on biosensors. The sensor particles were identical to the ones measured with the BioZ^o system in paragraph 3.7.2, though a different sensor layout was chosen where the particles are directly immobilized on titanium substrates.

Nicotine MIP and NIP samples were mounted into single-channel liquid cells setups discussed in paragraph 2.2, after which these cells were filled with an amount of 200 μ l of PBS at pH 7. After about 1500 s of stabilization time an increasing concentration of target molecule was added at 1800 s intervals.



Figure 4-8: Impedance magnitude (top) and phase (bottom) versus frequency of the directly immobilized MIP sample for pure PBS and a concentration of 100 nM of nicotine

Figure 4-8 shows the magnitude (top) and phase (bottom) of the MIP impedance at pure PBS and 100 nM of nicotine concentration. One can distinguish a capacitive component in these plots, as shown by the dropping magnitude at increasing frequency and a negative phase shift. A resistive component is also present in series with this capacitance, as the phase approaches zero at high frequencies and the magnitude stabilizes. This resistive component is most likely due to the resistivity of the solution, substrate and electrodes. It should be noted that a separate resistive component is commonly present in parallel with the interfacial capacitance, as was shown in paragraph 2.1. This can also be seen in the low frequency part of these graphs, were the phase drops towards zero and a slight decrease in slope is present in the magnitude.

Response in the sensor layer was determined to be most dominant at a frequency of 200 Hz, thus in the most capacitive part of the impedance. The calculated capacitance value drops from 270 nF, at pure PBS, to 218 nF for the 100 nM nicotine. In figure 4-9 the time-resolved magnitude is plotted at this frequency for pure PBS and a concentration of 10 and 20 nM. Addition of 10 nM causes no distinguishable impedance shift, indicating that this concentration is below the detection limit of the sensor. At 20 nM however a significant shift in impedance was measured, after which the signal again stabilized.



Figure 4-9: Impedance magnitude versus time for the directly immobilized MIP sensor at pure PBS, 10 nM and 20 nM of nicotine

The small dips in impedance that can be seen at addition are a result of short temperature fluctuations. Measurements were repeated on a complete concentration range of 10 nM to 100 nM and a concentration curve was plotted to gain insight in overall sensor performance. Figure 4-10 shows the differential sensor response in respect to the value after stabilization in PBS. Although the sensor was fabricated similarly as the one discussed in figure 4-9, a concentration of 10 nM lies well above the detection limit, even resulting in a response of more than 2 %. The sensor saturates at a concentration of 50 nM. This difference in performance in respect to figure 4-8 could be due to issues in the still experimental and manually labor intensive fabrication procedure involved with this sensor layout. Nevertheless this concentration curve shows the possibilities for detection of concentrations in nM range using the presented BioZLCD system.



Figure 4-10: Concentration curve, showing relative impedance magnitude response versus an applied concentration of target molecules, for a directly immobilized nicotine MIP sensor

4.6 Result and discussion

In this chapter the development of a stand-alone, miniature biosensor impedance analyzer was documented. Impedance measurement circuitry was kept identical to the previously described BioZ^o system while a central ARM-based processor runs the measurement routine and provides ways for data processing. An internal battery and numerous user communication protocols enable application in different settings. Special care was taken to avoid interference of the added digital subcircuit on the measurement circuitry. An RTOS running different tasks, e.g. for data storage and the web server, ensures stable operation of the system. The completed device is shown in figure 4-11 together with a 1-euro coin for size reference. The circuit diagrams and bill of materials of this device are present in appendix 4.4.



Figure 4-11: BioZLCD impedance spectroscopy unit

All connectors on the BioZLCD system including coaxial SMBs for the four measurement channels and custom peripheral ports are present on the back of the device. The touch screen display is mounted in the sloped top of the enclosure for ease-of-control.

Measurements performed on passive components indicate similar though slightly more accurate performance as the BioZ° device. An average impedance magnitude error of 0.22 % was achieved within in the complete range of 10 Ω to 1 M Ω . Biomimetic MIP sensor response near the detection limit up to saturation of the sensor could be monitored.

The presented BioZLCD system could prove useful in point-of-care and field settings were space is limited, no power supply is present or a high level of user control is required. Examples include the use in hospitals and environmental monitoring settings.

Chapter 5

Smartphone based biosensor readout

"iBioZ"

When looking at the large market of consumer-friendly glucose sensors and pregnancy tests it becomes clear that biosensors can be relevant in commercial consumer settings. Furthermore, the biomimetic MIP sensors discussed in the previous chapter inhibit detection limits at very low concentrations (in the order of several nM) and high specificity combined with robustness and long shelf life, making them viable in commercial healthcare applications. This chapter presents a possible solution for MIP readout in consumer setting based on smartphone technology combined with disposable test strips.

5.1 Design concept and system overview

When developing devices specifically for the consumer market, specific design factors come into play. According to Dieter Ram's celebrated ten principles of good design [137], a well-designed consumer product should be:

- Innovative
- Usefull
- Aesthetic
- Understandable
- Unobstrusive
- Honest
- Long-lasting
- Thorough down to the last detail
- Environmentally friendly
- As little design as possible (focus on simplicity)

Biosensors could penetrate a consumer market much more easily if they could be read out by means of existing consumer devices, preferably with as little external hardware as possible. Ideally, suitable devices should meet the ten discussed standards while incorporating extensive processing power for measurement analysis, communication protocols for remote monitoring and some highly visual way of displaying results. One possible solution can be found in the smartphone and tablet PC market.

Development of current-generation smartphone technology began with the release of the first Apple Inc. iPhone in 2007. This device was in essence a combination between a mobile phone, a personal digital assistant (PDA) and an iPod touch music player. The success of the iPhone triggered the development of similar smartphones and tablet PCs from a wide range of manufacturers. It is a general consensus to differentiate between smartphones based on the operating system (OS), the most common being Google Inc. Android OS, Windows Co. Mobile and Apple Inc. iOS. The choice was made to develop for the Apple iOS operating system, thus restricting device choices to Apple iPhone smartphone and iPad tablet PC.

As was shown in previous chapters, impedimetric readout of biosensors is commonly performed within a frequency spectrum of 10 Hz to 100 kHz. The ideal frequency is strongly dependent on the specific sensor purpose and target, though for the developed and tested sensor setups these frequencies lie well within the audio spectrum of 20 Hz to 20 kHz. The amplitude of the excitation voltages was set at 65 mV for the developed BioZ^o and BioZLCD measurement devices. Last but not least, biosensor measurements are rarely performed over a single channel. It is usually required to measure at least one reference channel together with the sensor, to rule out side effect like temperature fluctuation and non-specific binding events. Examples of this principle can be found in the MIP/NIP sensor readout in chapter 4.

The standard audio outputs of smartphones, normally used for headphones, are well suited for these potentials within the desired frequency range. The left and right audio channel can be used for two-channel signal output. After conversion to a voltage the resulting current caused by the impedance of a sample can be read by the microphone input of the device. Since both a 2-channel audio output and a microphone input are commonly present on the same 4-pole audio jack, this allows for miniature, user-friendly impedimetric biosensor readout setups.

The maximum sample rate of the audio in/output of the chosen devices lies at 44.1 kHz with a bit rate of 16 bit. This puts some limitations on the impedance and frequency resolution and range, which will be taken into account during design and performance verification. It should be noted that the impedance chip inside the BioZ^o and BioZLCD analyzers has a resolution of merely 12 bit. However, due to the nature of these devices it was possible to incorporate external circuitry to divide the relatively large impedance range into subranges, each utilizing the full 12 bit resolution. Since external hardware and component

cost is kept to a minimum in the smartphone-based analyzer this was not an option.

When developing devices for a wide range of biosensors, one would ideally have a low-cost, compact device in which different types of disposable sensors can be mounted, e.g. in the form of test strips. This approach was for example taken with the OneTouch and Accu-Chek glucose meters [5, 138]. A test strip was designed consisting of 2 working electrodes and a single counter electrode. Hardware was designed to convert the smartphone audio signals to enable impedance spectroscopy on this test strip.



Figure 5-1: Smartphone based biosensor readout concept

The audio signals are completely controlled by means of a mobile application (app) running on the smartphone or tablet PC. This app also allows for automated data analysis and displays results in a highly graphical manner. Figure 5-1 shows a concept drawing of the system, which was named iBioZ. BioZ indicates the ability of measuring biosensor impedances while "i" is a commonly used internet-related prefix, more specifically for current generation multimedia devices. The following paragraphs give a detailed overview of the different design aspects of this system.

5.2 Hardware implementation

Since excitation signal generation and processing the resulting voltages is entirely done by respectively the audio output and microphone input of the smartphone, only a minimum of external hardware is required. The developed hardware is shown in Figure 5-2. Power supply circuitry has been left out of this image for reasons of clarity.



Figure 5-2: Circuit diagram of the hardware developed for interfacing smartphone signals to a test strip

The 50 mV sine wave, alternately generated by the left and right channel audio output, is buffered by means of an Analog Devices AD8030 dual OPAMP. This OPAMP has a wide operating voltage range with a relatively low power consumption of 1.3 mA, making it suitable in low-power devices. The buffered signals of both channels are subsequently connected to either a test strip, mounted in a Samtec card edge connector, or shielded coaxial cables.

An AD820 inverting OPAMP circuit converts the resulting current, caused by the impedance of an attached sample, back to a voltage. The OPAMP was again chosen for its wide operating voltages and low quiescent current of merely 800 µA. The resulting voltage can be read by the microphone input of the smartphone, though it should be noted that this input has a maximum of about 42 mV. Since the samples behave like input resistors of the inverting OPAMP circuit, operating at a fixed input voltage of 50 mV, the feedback resistor of this circuit was chosen slightly lower than the lowest measurable impedance. This causes the OPAMP circuit to always attenuate voltages, thus keeping the voltage received by the microphone input below 42 mV. As was shown in the previous chapters, biosensors typically operate in an impedance range of 100 Ω to 100 k Ω . A feedback resistor of 100 Ω was thus chosen. Since the iPhone and iPad microphone inputs both have a 16-bit resolution, voltage differences of 0.64 μ V can be detected. At the highest sample impedance of 100 k Ω the 50 mV audio output signal is attenuated 1000 times, resulting in a 50 μ V signal. Thus, even at the highest sample impedance, a measurement accuracy of almost 1 % would theoretically be possible.

An impedance matching circuit is needed for the smartphone to detect the circuit as an external microphone. This required a resistor in the range of 1 k Ω to 100 k Ω to be attached parallel to the microphone input. Furthermore, DC voltages were measured at this input. A 10 μF non-polar capacitor was used to avoid influence of unwanted voltages on the measurement.

Power is supplied to the circuit via two 3 V, 140 mAh CR1632 batteries (not shown in the circuit diagram). The operating time was rated at about 70 hrs. The +/-3 V battery supply is the only supply voltage present in the system and these voltages are only used to power the OPAMPs. A 4-layer PCB layout was chosen where an analog ground, being the ground of the batteries, in used as an inner layer for the largest part of the PCB surface. The digital smartphone/tablet PC ground present on the audio jack is connected to the analog ground as close as possible to the jack to avoid digital interference from the device. Furthermore, decoupling capacitors of 100 nF and 10 nF in 0402 SMD package were placed at the OPAMP supply voltage pins as a manner for noise reduction.

5.3 Software methodology

All software runs on the smartphone/tablet PC used for the measurements in the form of an app. This app was developed by means of Apple Inc.'s Xcode software development kit (SDK), using the proprietary objective-C based Cocoa Touch application programming interface (API). Figure 5-3 shows a screenshot

of the user interface when running the app on an iPad tablet PC. This section discussed the measurement routine while briefly addressing the frameworks and methods used in development of the software.



Figure 5-3: Screenshot of the software running on an iPad tablet PC

The biosensor readout app was written around a Model-View-Controller architecture, as is a common technique in iOS app development. Views are drawn in a storyboard, depicted for the discussed iPhone app in figure 5-4, and linked via outlets and actions to a controller. This controller interacts with models, e.g. objects containing data.

Upon starting the app the user can either perform a sweep in a chosen frequency spectrum or perform time resolved measurements at a calculated frequency. The user can also choose to view the history of previous measurements. This is done in the "Settings" view, which is linked to the Settings View Controller. In this controller parameters are calculated using the settings chosen by the user, e.g. an array is calculated containing the different frequencies in a set spectrum. Upon starting a measurements a segue occurs towards the Graph View where the current measurement is displayed. If the

user chose to view the previous measurements a segue towards the History View is performed instead. Here the user can select a previous measurement from a table, after which a segue is again done towards the Graph View in order to display this measurement.



Figure 5-4: iPhone storyboard for the presented app

If the user opted to perform time resolved measurements a Bode plot is first measured for both channels to calculate the ideal measurement frequency. An array of frequencies, logarithmically divided between 20 Hz and 20 kHz is created, after which each frequency is separately generated at the audio output at a fixed peak-to-peak amplitude of 50 mV. A 1024-samples Fast Fourier Transform (FFT) is subsequently performed on the values in the microphone input buffer to determine the input amplitude at the current output frequency. Impedance is calculated from the ratio of the measured input amplitude, the fixed output amplitude and the known feedback resistor of the I-V conversion OPAMP. This measurement routine is executed by a dedicated class which functions as a delegate for the Graph View Controller. This way the Graph View Controller is only responsible for handling interface events such as zooming and scrolling in the graph, avoiding the risk for interface blocking and enhancing user experience. Figure 5-5 gives an overview of the code sequence while the

blue color indicates the use of specific frameworks other than the user interface manager UIKit.



Figure 5-5: Simplified iOS code flowchart

The choice was made to implement the open-source Core Plot framework for relatively easy plotting of the time-resolved measurements in a graphically attractive manner. This framework was thus implemented in the Graph View Controller class. All audio frameworks within the Cocoa Touch API are included in the Core Audio infrastructure. Since the app will be handling low-level audio data, i.e. outputting and reading sine waves with specified amplitude and frequency, the choice was made to implement Audio Unit plugins, the lowest level of audio framework available. A methodology was chosen where a single unit is responsible for both the output of samples as reading the buffers of input data. To do so, two different callback structures are used which run quasi-simultaneously, as is documented in [139]. An output callback fills the output buffers of both channels with the desired amplitude value for each sample, using equation 5.1.

$$A_n = \sin \frac{n \times 2 \times \pi \times f}{44100} \tag{5.1}$$

Here A_n is the amplitude for sample n, with f being the frequency of the output sine wave. The sample rate is fixed at 44.1 kHz. The input callback fills a 2048 byte buffer with 1024 16 bit values, being 23 ms of audio input data. This audio data is subsequently converted to floating point numbers between 0 and 1 by dividing each sample by 32768. To allow for stabilization of the excitation signal when measuring electrochemical biosensor measurement cells, a delay of 100 ms is implemented between signal output and buffer readout. The vDSP API, contained within the Accelerate framework, provides methods for performing Fourier transforms. A Hanning window is applied to the samples data after which the data is converted to split-complex format, as is required by the build-in FFT functions. A 1024-point FFT is then performed.

After the completion of the spectrum measurement the ideal frequency for timeresolved measurement and plotting is calculated. As was shown in the previous chapters, the maximum impedimetric response upon target molecule detection in biosensors can be commonly seen in the capacitive interface between solid sensor layer and analyte. The frequency at which this capacitance is most dominantly present is calculated using the slope of the magnitude plots. The magnitude of the frequency with maximum slope is subsequently measured continuously at intervals of 1 s to monitor response of the sensor.

5.4 System evaluation

The microphone input of both the iPhone smartphone and iPad tablet PC devices was shown to possess band pass filtering behavior. When performing measurements without compensation for this filter the usable frequency range, i.e. the pass band of the filter, is limited from 200 Hz to 9 kHz. It is possible to compensate for this filter in hardware design, though this would make the hardware platform dependent. A frequency dependent scaling factor was instead implemented in software. Calculated impedances are multiplied by the factor to compensate for the filtering behavior. Figure 5-6 shows a measurement on a 150 Ω and 2.2 k Ω resistor both with and without compensation by means of this factor. When applying the filter compensation the measured magnitude approaches a constant value in respect to the frequency. The 2-channel audio output of the devices was verified to inhibit no filtering behavior within the audio spectrum.



Figure 5-6: Impedance magnitude versus frequency with and without compensation for the microphone input filter of iPhone and iPad devices

The accuracy of the system was analyzed by measuring a set of metal-film resistors from the E12 series within the range of 100 Ω to 100 k Ω . Measurements were performed at a fixed frequency of 2 kHz, thus in the center of the audio spectrum. Deviation was calculated in respect to the values measured with a Keithley 2000 digital tabletop multimeter. Fig. 5-7 shows the deviation versus the resistor value for the two measurement channels on the setup.



Figure 5-7: Relative impedance magnitude error versus impedance magnitude measured on a set of resistors at a fixed frequency of 2 kHz

The lowest measurable resistivity is about 120 Ω , corresponding to a microphone input voltage of 42 mV, as calculated in the previous sections. Values in between this minimum and a value of 10 k Ω have a deviation below 1 %. The deviation at higher resistivity increases however significantly, reaching 5 % at the aimed maximum impedance of 100 k Ω . This is most likely due to decreasing signal-to-noise ratio, thus lowering the accuracy achieved in the FFT. At a resistivity of 100 k Ω the 50 mV output signal results in a current of 500 nA, making the signal sensitive to noise. A magnitude difference of about 0.15 % can also be seen between the two channels. This is most likely due to losses in PCB traces, as the shielded cables and connectors were identical for both channels.

As a next step in performance analysis a previously discussed equivalent circuit of a diamond-based DNA denaturation measurement cell, shown in figure 3-12, was measured using an iPad tablet PC. Figure 5-8 shows the magnitude plot for this circuit in respect to the values measured with the BioZ^o device. Furthermore, a directly immobilized MIP sensor on a Ti substrate, as discussed in paragraph 4.5, was mounted in a static addition setup. The measurement cell was filled with PBS after which the magnitude plot was measured and again compared to values measured with the BioZ unit.



Figure 5-8: Impedance magnitude versus frequency for a MIP sensor and equivalent Randles circuit of a DNA setup in respect to the BioZ^o device

Both the equivalent Randles circuit of the DNA-denaturation setup as the MIP sensor follow similar magnitude plots when measured with the newly developed system and the BioZ^o device. It is thus possible to get an adequate indication of the magnitude versus frequency behavior of different biosensor setups. This magnitude data can be used to calculate the ideal measurement frequency in software, i.e. the frequency at with the maximum slope is reached and the interfacial capacitance is dominant.

5.5 Sensor strip readout

A test strip was developed in which both a MIP sensor as a NIP reference can be measured quasi-simultaneously in the same analyte. An electrode layout was chosen were MIP and NIP particles were immobilized on top of two working electrodes and a single uncovered counter-electrode is used.



Figure 5-9: Disposable sensor strip (left) and hardware with test strip mounted (right)

The developed sensor strip, shown in figure 5-9 both unmounted (top) and mounted in the developed hardware (bottom), consists of a custom sized 3 mm thick Teflon strip on which a poly(ethylene terephthalate) (PET) foil with screen printed electrodes is glued. A semi-automated Isimat 1000P screen-printing machine was used to print three lines of Dupont 5064 conductive silver ink on the PET foil at an interspacing of 0.8 mm. The resulting electrodes, which measure 2 mm by 20 mm in size and are approximately 50 μ m thick, have a resistivity of about 1 Ω .

Before actually immobilizing a sensor layer on the test strip, the capabilities of the developed impedance spectroscopy system in combination with the sensor strip were verified. A 2 mm thick O-ring with diameter of 7.3 mm was attached on the electrode strip by means of vacuum grease and functions as a liquid reservoir. Varying ion concentrations of PBS buffer liquid were injected in the liquid reservoir, after which impedance was measured. Figure 5-10 shows the magnitude plots for three dilutions in demineralized water of this buffer solution. An increasing magnitude can be seen at lower ion concentrations. These results are consistent with data found in literature [93], indicating correct impedimetric readout of the test strips in liquid media.



Figure 5-10: Impedance magnitude versus frequency for three dilutions of PBS on the test strip

A MIP based histamine sensor, similar to the sensor analyzed in paragraph 3.7.2, was immobilized on the electrode strip. A layer of about 100 nm of MDMO-PPV was spin-coated, after which MIP and NIP particles were stamped and baked onto the two outer PPV-covered electrodes. The center electrode remained covered solely with PPV. An amount 60 μ I PBS was placed inside the O-ring attached to the test strip, after which the O-ring was covered with a PET-foil to avoid evaporation. Magnitude plots were measured for both the MIP and the NIP electrode, as shown in figure 5-11.

The sensor was allowed to stabilize for 50 minutes before histamine was added up to a total target molecule concentration of 1 μ M. Time resolved impedance measurements were continuously performed at a fixed frequency of 500 Hz. Figure 5-12 shows the magnitude change relative to the initial magnitude upon addition of the histamine. An impedance magnitude increase in the MIP sample occurs after this addition, after which the signal again stabilizes. The NIP reference fluctuates slightly, possibly due to sudden temperature changes or liquid mobility caused by manually addition of the histamine liquid. However, the NIP stabilizes again at a value close to the initial value. A magnitude difference of about 150 Ω due to target molecule detection thus occurs in the differential MIP-NIP signal.


Figure 5-11: Impedance magnitude versus frequency for the MIP and NIP sample immobilized on the sensor strip



Figure 5-12: Impedance magnitude change of the MIP and NIP channel versus time upon addition of 1 μ M of target molecule

5.6 Towards home diagnostics applications

The previous paragraph has shown that the low-cost, smartphone based analyzer allows for accurate readout of custom-made MIP test strips. In order to use the system in a home diagnostics setting however, specific factors that could intervene with reliable functionality and accurate readout of the measurements come into play. The test strips behave as an electrochemical cell and as was already addressed in previous chapters, temperature fluctuation can cause significant changes in measured impedance. Furthermore, when using the smartphone system thermal fluctuations due to handling and movement of the device can reach values of several degrees centigrade. This can thus severely hinder readout and is especially an issue when measuring low sensor response. To demonstrate this, the sensor strip was exposed to thermal fluctuations using the external PID-based heating unit discussed in paragraph 3.2. A random generator was used in the PID control to induce temperature noise between 25 °C and 27 °C at 30 s intervals. A Pt100 resistance thermometer was measured together with a sensor strip containing solely MIP particles. Figure 5-13 shows the effect of the measured temperature fluctuations in respect to the average value of 26 °C (red), together with the measured impedance magnitude of the MIP sensor (blue).



Figure 5-13: The effect of temperature noise on the measured impedance magnitude versus time upon addition of a low concentration of target molecule

The measured magnitude behaves inversely proportional to the temperature changes, in accordance with figure 3-15, while the addition of a low concentration of 20 nM histamine seems undetectable.

The temperature noise was calculated to be -70 Ω /°C. It should thus be noted that even at relatively high concentrations of target molecules, as is the case in figure 5-10, temperature fluctuations of several degrees could significantly raise the detection limit. Since the effect of the temperature noise on the impedance can be easily quantified, it is possible to filter these fluctuations in the measurement software using a reference temperature sensor or the NIP sensor. This latter is already commonly used as a reference to rule out other, much less significant environmental noise sources such as vibrations. Figure 5-14 shows the magnitude signal of figure 5-13 before (red) and after (blue) removal of the temperature-induced noise using the calculated ratio. The low concentration of 20 nM of target molecule can now be clearly detected.



Figure 5-14: MIP impedance magnitude response versus time before and after removal of temperature-induced noise

The remaining noise level lies at about 20 Ω , being similar to the noise present in the MIP signal of figure 5-12. The total response at this concentration is about 50 Ω , thus well above the present noise level. Furthermore, basic algorithms, such as a moving average filter, can easily be implemented in the app to further reduce this noise if deemed necessary for specific applications.

5.7 Result and discussion

In this chapter a way of biosensor readout using the on-board audio hardware of smartphones and tablet PCs was presented. The required external hardware is kept to a minimum to reduce cost and maintain portability, while intuitive user control and automated data processing are possible by means of an app. The presented iBioZ setup including the app running on an Apple iPhone 5, the external hardware connected via standard audio cable and a 2-channel MIP test strip mounted is shown in figure 5-15. In appendix 4.5 a circuit diagram and bill of materials of this external hardware can be found.



Figure 5-15: iBioZ system

The specifications of the presented system are limited when compared to the previously discussed BioZ° and BioZLCD devices. Since audio signals are used for impedance measurements the frequency range is fixed from 20 Hz to 20 kHz. The exact impedance range is dependent on the feedback resistor R1 in figure 5-2, though it is limited to 2 decades if measurement errors of less than 1 % are required. Nevertheless the system is capable of performing accurate impedance spectroscopy measurements within frequency and impedance magnitude ranges typical for bio(mimetic)sensors. Algorithms can be implemented in software to further enhance accuracy and rule out external

influences. One example was given where temperature noise is ruled out by means of a reference channel.

Small interface modifications in the app can result in a simple to understand true/false indication of specific target molecule concentrations present in a biological liquid, similar to existing pregnancy tests. Since the evaluated sensor type has already been used with success to detect target molecules in saliva [140] and blood plasma using MIP based sensors [42], the system can prove useful in home-monitoring applications. Furthermore, the amount of liquid used in the discussed measurements is as low as 60 μ l and liquid deposition by the user can be simplified in further development towards the level of glucose meters and pregnancy tests.

Chapter 6

General conclusion and future outlook

First prototypes for three different readout devices were presented in this work, each targeting a specific biosensor application. As was shown in the introductory chapter, biosensors have been around for decades and are continuously being innovated. The range of detectable biologically and medically relevant molecules keeps steadily increasing. The second chapter gave an overview of the chosen measurement technique, being impedance spectroscopy, and the materials used for applying this technique in biosensor readout. In the third chapter the design steps taken to develop a miniature impedance analyzer specifically fine-tuned for biosensor characterization in the lab were thoroughly explained. The fourth chapter gave an overview of the steps taken to fine-tune this device for field use. Last but not least, a smartphone based readout mechanism using disposable test strips was demonstrated in chapter 5. Performance of the presented devices was verified on passive components and biomimetic MIP sensors. High enough readout accuracy is achieved in these devices to quantize sensor response at very low concentrations, i.e. in the nM range. As impedance spectroscopy is one of the most used biosensor readout techniques, this could greatly ease the readout of a broad range of biosensors. Table 6-1 gives an overview of the presented readout devices, characteristics and target applications.

	BioZ°	BioZLCD	iBioZ
Frequency spectrum	10 Hz - 100 kHz	10 Hz - 100 kHz	20 Hz - 20 kHz
Impedance range	10 Ω – 1 MΩ	10 Ω – 1 MΩ	120 Ω – 100 kΩ
Channels	8	4	2
User control	PC	Touchscreen/PC	Smartphone
Intended application	Lab setups	Field use	Consumer

Table 6-1 Overview of the presented devices

This thesis shows that it is possible to develop readout devices targeting a specific biosensor application. These devices were designed with a focus on low cost, small size and ease-of-use while achieving high accuracy and broad readout ranges. The applications of the BioZ^o device could however be extended well beyond biosensors, as this device could be used in general multichannel impedance spectroscopy setups. The limiting factors in this device are the excitation voltage level and frequency range, which are fine-tuned for biosensing purpose. However, the 10 Hz to 100 kHz spectrum could be for example used in audio applications and similar settings. The design concepts presented in the stand-alone BioZLCD device can be taken as a guideline for increasing the user interfacing of general lab readout equipment. Extending the functionality of lab equipment with touchscreen, local memory and wireless communication can form a basis for commercial devices.

The presented devices are hand-held in size, external equipment is however often needed for adequate sensor readout. Biological material is commonly kept at body temperature during measurements. Mechanisms for automatic, high accuracy analyte dosage are also often present in these setups. Future research can lead towards completely miniaturized measurements systems, up to microfluidics and micro-thermo-dynamics level. It should be noted that the requirements for the purely synthetic MIP based sensors, discussed in measurements throughout this work, are much less strict since sensing is not based on a biological layer. This should make the smartphone based readout system in combination with MIP test strips, including a reference NIP sample, viable in consumer settings.

Although this work is completely based on impedance spectroscopy as readout technique, the design strategies used can be converted towards other readout mechanisms. Recent advances include using the thermal resistivity of the biosensor layer for monitoring response. Similar to impedance spectroscopy this technique combines electronic readout with a non-invasive character and has been used with success for DNA SNP detection [29] and MIP readout [141]. Future work could include porting the presented devices for this recently discovered measurement technique. A basic principles for smartphone-based thermal biosensor readout was already presented in [142]. Also the combination between impedance spectroscopy and other readout techniques could prove useful in biosensor characterization setups. As the BioZ° and BioZLCD units have custom peripheral ports available they inhibit the possibility to interface with lab equipment, including other readout devices. Last but not least the presented device could be used in applications other than biosensor readout. Impedance spectroscopy is used in numerous non-healthcare related industrial settings, including battery characterization [143], solar cell analysis [144] and oil quality testing [145]. The compact, user-friendly systems presented in this work can be a major improvement in respect to the commercial devices currently used in these settings.

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Appendix 1: publications and conference contributions

International publications

- <u>J. Broeders</u>, D. Croux, M. Peeters, T. Beyens, S. Duchateau, T.J. Cleij, P. Wagner, R. Thoelen, W. De Ceuninck, Mobile application for impedance based biomimetic sensor readout, IEEE Sensors 13, 2659-2665 (2013)

- S. Duchateau, J. Broeders, D. Croux, D. Janssen, J.-M. Rigo, P. Wagner, R. Thoelen and W. De Ceuninck, Cell proliferation monitoring by multiplexed electrochemical impedance spectroscopy on microwell assays, Physica Status Solidi (c) 10, 882-888 (2013)

- D. Croux, T. Vangerven, <u>J. Broeders</u>, M. Peeters, S. Duchateau, J. Boutsen, W. Deferme, P. Wagner, R.Thoelen, W. De Ceuninck, Disposable wireless MIP-based biosensor tags, Physica Status Solidi (a) 210, 934-944 (2013)

- M.S. Murib, B. van Grinsven, L. Grieten, S.D. Janssens, V. vermeeren, K. Eersels, <u>J. Broeders</u>, M. Ameloot, L. Michiels, W. De Ceuninck, K. Haenen, M.J. Schöning and P. Wagner, Electronic monitoring of chemical DNA denaturation on nanocrystalline diamond electrodes with different molarities and flow rates, Physica Status Solidi (a) 210, 911-917 (2013)

- J. Broeders, D. Croux, A. Weustenraed, W. Vanaken, S. Duchateau, T.J. Cleij, P. Wagner, R. Thoelen and W. De Ceuninck, Embedded unit for point-of-care impedimetic biosensor readout, Proceedings of the IEEE 14th International Conference on High Performance Computing and Communications, 1571-1577 (2012)

- J. Broeders, S. Duchateau, B. Van Grinsven, W. Vanaken, M. Peeters, R. Thoelen, T. Cleij, P. Wagner and W. De Ceuninck, Miniaturized eight channel impedance spectroscopy unit as sensor platform for biosensor applications, Physica Status Solidi (a) 208, 1357-1363 (2011)

Oral presentations

- Engineering of Functional Interfaces 2013, Hasselt (Belgium) 8-9/07/2013, Thermal readout system for DNA polymorphism detection, <u>J. Broeders</u>, K. Eersels, M.S. Murib, D. Croux, R. Merken, B. van Grinsven, P. Wagner, R. Thoelen and W. De Ceuninck.

- Engineering of Functional Interfaces 2013, Hasselt (Belgium) 8-9/07/2013, Further advances in disposable wireless MIP-based sensor tags, D. Croux, <u>J.</u> Broeders, K. Van Den Bossche, P. Wagner, R. Thoelen and W. De Ceuninck.

- Engineering of Functional Interfaces 2013, Hasselt (Belgium) 8-9/07/2013, Arbitrary wave electrochemical impedance spectroscopy, a fast and reliable measurement technique, S. Duchateau, <u>J. Broeders</u>, D. Croux, P. Wagner, R. Thoelen and W. De Ceuninck.

- Engineering of Functional Interfaces 2012, Zweibrücken (Germany) 16-17/07/2012, Embedded unit for point-of-care biosensor readout, <u>J. Broeders</u>, D. Croux, A. Weustenraed, T.J. Cleij, P. Wagner, R. Thoelen and W. De Ceuninck.

- Engineering of Functional Interfaces 2012, Zweibrücken (Germany) 16-17/07/2012, Using impedance spectroscopy for in-situ cell growth monitoring, S. Duchateau, <u>J. Broeders</u>, D. Croux, M. Daenen, P. Wagner, J.M. Rigo, W. De Ceuninck and R. Thoelen.

- Engineering of Functional Interfaces 2012, Zweibrücken (Germany) 16-17/07/2012, Disposable wireless MIP-based sensor tags, D. Croux, <u>J. Broeders</u>, T. Vangerven, M. Peeters, J. Boutsen, W. Deferme, P. Wagner, R. Thoelen and W. De Ceuninck.

- IEEE 9th International Conference on Embedded Software and Systems, Liverpool (UK) 25-27/06/2012, Embedded unit for point-of-care impedance based biosensor readout, <u>J. Broeders</u>, D. Croux, A. Weustenraed, W. Vanaken, S. Duchateau, T.J. Cleij, P. Wagner, R. Thoelen and W. De Ceuninck.

- Graduate student Symposium on molecular imprinting, Imperial College London (UK) 28-30/09/2011, Surface molecular imprinting as versatile tool for thin-layer sensor applications, A. Weustenraed, F. Horemans, H. Diliën, D. Croux, J. Broeders, R. Thoelen, L. Lutsen, D. Vanderzande and T.J. Cleij.

- Graduate student Symposium on molecular imprinting, Imperial College London (UK) 28-30/09/2011, Newest advances in MIP biosensor readout, D. Croux, <u>J. Broeders</u>, A. Weustenraed, W. Vanaken, F. Horemans, P. Wagner, W. De Ceuninck and R. Thoelen

- Engineering of Functional Interfaces 2011, Linz (Austria) 19-20/07/2011, Miniaturized measurement setup for biosensors: further progress towards labon-a-chip, <u>J. Broeders</u>, D. Croux, J. Alenus, A. Weustenraed, F. Horemans, T.J. Cleij, P. Wagner, R. Thoelen and W. De Ceuninck.

- Engineering of Functional Interfaces 2011, Linz (Austria) 19-20/07/2011, Using impedance spectroscopy for in-situ cell growth monitoring, S. Duchateau, D. Janssen, <u>J. Broeders</u>, M. Daenen, P. Wagner, J.M. Rigo, W. De Ceuninck and R. Thoelen.

- Engineering of Functional Interfaces 2010, Marburg (Germany) 15-16/7/2010, Impedance based DNA Spectroscopy, S. Duchateau, J. Broeders, P. Wagner, R. Thoelen and W. De Ceuninck.

Poster contributions

- 7th International Conference on Molecularly Imprinted Polymers – Science and Technology, Paris (France) 27-30/08/2012, Surface molecular imprinting as a versatile tool for thin-layer sensing, A. Weustenraed, F. Horemans, H. Dilliën, D. Croux, J. Broeders, M. Peeters, R. Thoelen, P. Wagner, T.J. Cleij.

- Biosensors 2012, Cancun (Mexico) 15-18/05/2012, Increasing MIP-sensor usability in field application, <u>J. Broeders</u>, D. Croux, A. Weustenraed, M. Peeters, W. Vanaken, S. Duchateau, T.J. Cleij, P. Wagner, R. Thoelen and W. De Ceuninck.

- UHasselt NanoSensEU Symposium, Diepenbeek (Belgium) 25/04/2012, Advances in label-free biosensor readout techniques, D. Croux, <u>J. Broeders</u>, S. Duchateau, A. Wuestenraed, W. Vanaken, T.J. Cleij, P. Wagner, R. Thoelen and W. De Ceuninck.

- 8th IBRO Congress of neuroscience, Firenze (Italië) 14-18/07/2011, Using impedance spectroscopy for monitoring the proliferative activity of microglial cells, D. Janssen, S. Duchateau, K. Verboven, <u>J. Broeders</u>, W. De Ceuninck, R. Thoelen and J.M. Rigo.

- BPS Meeting 2011, Namur (Belgium) 25/05/2011, Miniature impedance spectroscopy setup: further progress toward lab on chip, D. Croux, <u>J. Broeders</u>, J. Alenus, A. Weustenraed, T. J. Cleij, P. Wagner, R. Thoelen and W. De Ceuninck.

- BPS Meeting 2011, Namur (Belgium) 25/05/2011, Using impedance spectroscopy for in situ cell growth monitoring, S. Duchateau, <u>J. Broeders</u>, K. Verboven, D. Janssen, P. Wagner, W. De Ceuninck and R. Thoelen.

- BNV-NNV Fysica 2010 Symposium, Utrecht (Netherlands) 23/04/2010, Electrochemical Impedance Spectroscopy, <u>J. Broeders</u>, S. Duchateau, P. Wagner, W. De Ceuninck and R. Thoelen.

Appendix 2: terminology

Ab	Antibody
ABS	Acrylonitrile-butadiene-styrene
ADC	Analog-to-digital converter
Ag	Antigen
API	Application programming interface
App	Mobile application
ARM	Acorn RISC processor
	Double laver capacitance
CHO	Chinese hamster ovary cell
CPE	Constant phase element
	C-reactive protein
	Digital-to-analog converter
DAC	Digital-to-analog converter
DDS	Direct digital synthesis
	Direct memory access
	Duibecco s Modified Eagle s Medium
DMM	
DNA	Deoxyribonucleic acid
DSP	Digital signal processor
ECG	Electrocardiogram
EDC	1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide
	hydrochloride
EEPROM	Electrically erasable read-only memory
EIS	Electrochemical impedance spectroscopy
FFT	Fast Fourier transformation
FPU	Floating point unit
FSMC	Flexible static memory controller
HEK	Human embryonic kidney cell
I ² C	Inter-integrated circuit bus
IC	Integrated circuit
ICG	Impedance cardiography
IP	Internet protocol
IAN	Local area network
LCD	Liquid crystal display
Li-Ion	Lithium-ion
MCU	Microcontroller unit
MIP	Molecularly imprinted polymer
NCD	Nano crystalline diamond
NTP	Non-imprinted polymer
	Operational amplifier
	Decembra buffered caline
	reisuidi ülyildi dssisidill Dolu(othulono toronbtholoto)
QCM	Quartz crystal microbalance
κ _p	Polarization resistance

R _{ct} RTD RTOS	Charge transfer resistance Resistive thermal device Real time operating system
SD	Secure digital
SDK	Software development kit
SEM	Scanning electron microscope
SMD	Surface mount device
SNP	Single nucleotide mismatch
SOC	System-on-a-chip
SPI	Serial peripheral interface bus
SPP	Serial port profile
SPR	Surface plasmon resonance
TFT	Thin film transistor
UART	Universal asynchronous receiver/transmitter
UDP	User datagram protocol
USB	Universal serial bus
Z	Impedance magnitude

Appendix 3: list of figures and tables

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Figure 1-2: Basic biosensor layout

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Figure 3-13: Relative impedance magnitude drift of an equivalent RRC circuit versus time

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Figure 3-16: Nyquist plots showing imaginary versus real impedance at different temperature levels for boron-doped diamond in PBS

Figure 3-17: Concentration curve displaying relative impedance magnitude change versus concentration for a MIP-based nicotine sensor

Figure 3-18: Relative differential MIP-NIP signal response versus time upon histamine addition

Figure 3-19: Real (top) and imaginary (imaginary) impedance of the PPVimmobilized MIP and NIP samples in respect to their initial values versus measurement time

Figure 3-20: Bode plots showing impedance magnitude and phase versus frequency for three types of cells commonly used in biomedical research Figure 3-21: Impedance magnitude change of different initial population sizes of BV2 cell versus time

Figure 3-22: BioZ° impedance spectroscopy unit

Figure 4-1: BioZLCD block diagram

Figure 4-2: ARM processor product range

Figure 4-3: LCD and microcontroller interfacing

Figure 4-4: BioZLCD PCB layout

Figure 4-5: RTOS tasks and measurement routine

Figure 4-6: Relative impedance magnitude error and phase error versus impedance magnitude

Figure 4-7: Equivalent circuit Bode plots, expressing impedance magnitude and phase versus frequency, as measured on the HP4194 and the BioZLCD unit Figure 4-8: Impedance magnitude (top) and phase (bottom) versus frequency

of the directly immobilized MIP sample for pure PBS and a concentration of 100 nM of nicotine

Figure 4-9: Impedance magnitude versus time for the directly immobilized MIP sensor at pure PBS, 10 and 20 nM of nicotine

Figure 4-10: Concentration curve, showing relative impedance magnitude response versus an applied concentration of target molecules, for a directly immobilized nicotine MIP sensor

Figure 4-11: BioZLCD impedance spectroscopy unit

Figure 5-1: Smartphone based biosensor readout concept

Figure 5-2: Circuit diagram of the hardware developed for interfacing smartphone signals to a test strip

Figure 5-3: Screenshot of the software running on an iPad tablet PC

Figure 5-4: Microphone input filter with and without compensation

Figure 5-5: Simplified iOS code flowchart

Figure 5-6: Impedance magnitude versus frequency with and without compensation for the microphone input filter of iPhone and iPad devices Figure 5-7: Relative impedance magnitude error versus impedance magnitude measured on a set of resistors at a fixed frequency of 2 kHz Figure 5-8: Impedance magnitude versus frequency for a MIP sensor and

equivalent Randles circuit of a DNA setup in respect to the BioZ° device

Figure 5-9: Disposable sensor strip and hardware with test strip mounted Figure 5-10: Impedance magnitude versus frequency for three dilutions of PBS on the test strip

Figure 5-11: Impedance magnitude versus frequency for the MIP and NIP sample immobilized on the sensor strip

Figure 5-12: Impedance magnitude change of the MIP and NIP channel versus time upon addition of 1 μM of target molecule

Figure 5-13: The effect of temperature noise on the measured impedance magnitude versus time upon addition of a low concentration of target molecule Figure 5-14: MIP impedance magnitude response versus time before and after removal of temperature-induced noise

Figure 5-15: iBioZ system

Table 3-1: Excitation voltages and related DC bias

Table 3-2: Impedance subranges for feedback and calibration resistors

Table 4-1: BioZ^o and BioZLCD performance

Table 6-1: Overview of the presented devices

Appendix 4: technical documentation

4.1 Single channel addition setup



4.2 4-channel flowcell





4.3 BioZ° circuit diagrams and bill of materials

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Designator	Quantity 1	Aanufacturer	Part Number	Value	PackageReference
C1, C3, C6, C9, C10, C11, C16, C17, C18, C22, C24	1	Aurata	GRM155R71C104KA88D	0.1uF	0402(1005)
C2, C4, C8, C12, C13, C14, C15, C19, C20, C21, C23, C25	2	temet	C0603C106M9PACTU	10uF	0603(1608)
C5	_	Aurata		1uF	0603(1608)
C7	-	Aurata	GRM155R71E103KA01D	10nF	0402(1005)
D1	-	Vational Semiconductor	LM336-2.5		TO-92
D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, D12, D13, D14, D15, D16, D17, D18, D19, D20, D21, D22, D23, D24	5	Aulticomp	1N4148W		SOD-123
025	_	P	NP		LED-0
D26	-	P .	NP		0603(1608)
11	-	AULTICOMP	2212BR-05G		SIP-5
12, 13	-	YCO Electronics/AMP	826629-3		SIP-3
J4, J5		YCO Electronics/AMP	826629-2		SIP-2
J6		AMTEC	IPS1-110-01-S-D		DIP-20
18	-	Vuerth Elektronik	61201021721		DIP-10
61	-	1P	NP		TP
J10	-	4P	NP		SIP-5
K1, K2, K3, K4, K5, K6, K7, K8, K9, K10, K11, K12, K13, K14, K15, K16, K17, K18, K19, K20, K21, K22, K23	0	DMRON	G6K-2F-Y		CUSTOM SMT
P1,P2		YCO Electronics	1-1337482-0		SMB
R1, R6	-	AULTICOMP	MC 0.063W 0603 1% 100K	100kOhm	0603(1608)
R2	-	AULTICOMP	MC 0.063W 0603 1% 220K	220kOhm	0603(1608)
R3	_	/ishay Sfernice	MC 0.063W 0603 1% 20K	20kOhm	0603(1608)
R4, R5	_	AULTICOMP	MC 0.063W 0603 1% 10K	10.0kOhm	0603(1608)
R7	_	AULTICOMP	MC 0.063W 0603 1% 2K2	2.20kOhm	0603(1608)
R8	-	AULTICOMP	MC 0.063W 0603 1% 15K4	15.4k0hm	0603(1608)
R9	_	AULTICOMP	MC 0.063W 0603 1% 249R	2500hm	0603(1608)
R10, R15		AULTICOMP	MC 0.063W 0603 1% 3K8	3.8kOhm	0603(1608)
R11	_	AULTICOMP	MC 0.063W 0603 1% 56K	56kOhm	0603(1608)
R12	-	AULTICOMP	MC 0.063W 0603 1% 39R	390hm	0603(1608)
R13	-	AULTICOMP	MC 0.063W 0603 1% 97R6	970hm	0603(1608)
R14	_	AULTICOMP	MC 0.063W 0603 1% 1K69	1.7kOhm	0603(1608)
R16	~	AULTICOMP	MC 0.063W 0603 1% 7K4	7.4kOhm	0603(1608)
R17	~	AULTICOMP	MC 0.063W 0603 1% 820K	820kOhm	0603(1608)
R18	-	AULTICOMP	MCHP03W8F1005T5E	10MOhm	0603(1608)
R19	-	AULTICOMP	MC 0.063W 0603 1% 19K1	19kOhm	0603(1608)
R20	_	AULTICOMP	MC 0.063W 0603 1% 120K	120kOhm	0603(1608)
R21	-	AULTICOMP	MC 0.063W 0603 1% 316K	313kOhm	0603(1608)
R22	_	AULTICOMP	MCHP03W8F1504T5E	1.5Mohm	0603(1608)
R23	_	AULTICOMP	MCHP03W8F3904T5E	3.8MOhm	0603(1608)
R24		AULTICOMP	MC 0.063W 0603 1% 150R	1500hm	0603(1608)
10		nalog Devices	AD5933YKSZ		SSOP-16
U2, U5	_	vnalog Devices	OP27GSZ		SOIC-8
U3		tecom	RS3-0505D		SIP-7
U4	_	AAXIM INTEGRATED PRODUCTS INC	DS1077		uSOP-8
U6, U8	-	Inalog Devices	ADR433BRZ		SOIC-8
U7		exas Instruments	OPA627AUE4		SOIC-8
U9, U10	_	exas Instruments	PCA9535PWR		TSSOP-16
Л		AMTEC	IPT1-110-01-S-D		DIP-20
]2		W	200-6311-9UN-1900		PGA11x11
2		AAATE (INT4 440 04 C N		
11		AMTEC	PT1-110-01-S-D		DIP-20
۲۲,۲۲,۴2,۲3,۲4,۲۷,۲8,۲9,۲1,۲1,۲1,۲1,۲1,۲12,۲13,۲14,۲12,۲15,۲16	9	YCU Electronics	1-133/482-0		SMB



4.4 BioZLCD circuit diagram and bill of materials

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Quantity	Designator	Part Number	Footprint	Manufacturer	Value
8	R18, R19, R20, R21, R22, R23, R24, R25	CRCW04024K70FKED	RESC1005L	Vishay/Dale	4.7 kOhms
39	C5, C6, C7, C9, C11, C13, C15, C16, C17, C18, C19, C20, C21, C22, C23, C24, C26,	GRM155R61A104KA01D	CAPC1005L	Murata	0.1 uF
	228, C29, C30, C35, C37, C38, C39, C41, C43, C45, C48, C50, C51, C53, C55, C57, C58, C59, C60, C64, C65, C66				
9	R3, R6, R8, R10, R29, R30	CRCW0402000020ED	RESC1005L	Vishay/Dale	0 Ohms
1	R16	CRCW04021K50FKED	RESC1005L	Vishay/Dale	1.5 kOhms
5	R11, R27, R31, R55, R63	CRCW0402100KFKED	RESC1005L	Vishay/Dale	100 kOhms
11	R2, R9, R28, R36, R40, R41, R48, R49, R52, R61, R62	CRCW040210K0FKED	RESC1005L	Vishay/Dale	10 kOhms
2	C27, C52	GRM155R71E103KA01D	CAPC1005L	Murata	10nF
1	R67	CRCW060315K4FKEA	RESC1005L	Vishay/Dale	15.4 kOhms
4	C12, C14, C33, C34	GRM1555C1H180GZ01D	CAPC1005L	Murata	18 pF
2	R4, R7	CRCW04021R00JNED	RESC1005L	Vishay/Dale	1 Ohms
1	C47	GRM155R60J105ME19D	CAPC1005L	Murata	1uF
2	R17, R66	CRCW04022K20FKED	RESC1005L	Vishay/Dale	2.20 kOhms
1	R15	CRCW0402100KFKED	RESC1005L	Vishay/Dale	100 kOhms
1	R56	CRCW0603220KFKEA	RESC1005L	Vishay/Dale	220 kOhms
2	R5, R37	CRCW04022K00FKED	RESC1005L	Vishay/Dale	2 kOhms
1	R26	CRCW040236K0FKED	RESC1005L	Vishay/Dale	36 kOhms
9	R14, R43, R44, R45, R46, R47	CRCW040247K0FKED	RESC1005L	Vishay/Dale	47 kOhms
2	C31, C32	GRM1555C1H6R8CZ01D	CAPC1005L	Murata	6.8 pF
1	R76	CRCW06031M50FKEA	RESC1608L	Vishay/Dale	1.5 Mohms
1	R70	TNPW06031K69BEEA	RESC1608L	Vishay/Dale	1.7 kOhms
1	R65	CRCW060310M0FKEA	RESC1608L	Vishay/Dale	10 MOhms
19	C2, C3, C4, C8, C25, C36, C40, C42, C44, C46, C49, C54, C56, C61, C62, C63, C67, C68, C69	GRM188R60J106ME47D	CAPC1608L	Murata	10 uF
1	C10	GRM188R60J106ME47D	CAPC1608L	Murata	10 uF
1	R74	CRCW0603120KFKEA	RESC1608L	Vishay/Dale	120kOhm
7	R38, R39, R42, R50, R51, R53, R54	CRCW0603150RJNEA	RESC1608L	Vishay/Dale	150 Ohms
1	R73	CRCW060319K1FKEA	RESC1608L	Vishay/Dale	19 kOhms
2	R12, R13	CRCW060322R0FKEA	RESC1608L	Vishay/Dale	22 Ohms
1	R58	CRCW0603249RFKEA	RESC1608L	Vishay/Dale	250 Ohms
1	R59	CRCW06033K83FKEA	RESC1608L	Vishay/Dale	3.8 kOhms
1	R75	CRCW0603316KFKEA	RESC1608L	Vishay/Dale	313 kOhms
1	R1	CRCW0603330RFKEA	RESC1608L	Vishay/Dale	330 Ohms
1	R68	CRCW060339R0FKEA	RESC1608L	Vishay/Dale	39 Ohms
4	R32, R33, R34, R35	CRCW040249R9FKED	RESC1608L	Vishay/Dale	49.9 Ohms
1	R60	CRCW060356K0FKEA	RESC1608L	Vishay/Dale	56 kOhms
1	R71	CRCW0603620RFKEA	RESC1608L	Vishay/Dale	620 Ohms
1	R72	TNPW06037K50BEEA	RESC1608L	Vishay/Dale	7.4 kOhms
1	R64	CRCW0603820KFKEA	RESC1608L	Vishay/Dale	820 kOhms
1	R69	CRCW060397R6FKEA	RESC1608L	Vishay/Dale	97 Ohms

Quantity	Designator	Part Number	Footprint	Manufacturer	Value
2	11, 12	742792609	INDC1608L	Wurth Electronics Inc	EMI/RFI Suppressors & Ferrites 30ohms @ 100Mhz
5	D1, D2, D3, D4, D5	HSMG-C150	CD1608-0603GREEN	Avago Technologies	Standard LED - SMD Green Diffused 572nm 8mcd
1	C1	TR3B227M004C0700	TC3528-1411	Vishay Sprague	220 uF
1	Y1	ABM3B-25.000MHZ-B2-T	ABM3B	ABRACON CORP	25 MHz
1	Y3	ABM3B-8.000MHZ-B2-T	ABM3B	ABRACON CORP	CRYSTAL 8.000 MHZ 18PF SMD
1	Y2	ABS07-32.768KHZ-7-T	ABS07	ABRACON CORP	CRYSTAL 32.768KHZ 7PF SMD
1	U7	BT-23	BT-23	Amped RF	SPP Bluetooth Module
1	17	DM3AT-SF-PEJM5	DM3AT-SF-PEJ	Hirose Electric Co Ltd	CONN MICRO SD R/A PUSH-PUSH SMD
13	K1, K2, K3, K4, K10, K11, K12, K13, K14, K15, K16, K17, K18	G6K-2F-Y	OMRON G6K-2F-Y	OMRON	Low Signal Relay
5	K5, K6, K7, K8, K9	G6K-2F-Y	OMRON G6K-2F-Y	OMRON	Low Signal Relay
1	871	S2B-PH-SM4-TB	BATJST	JST	CONN HEADER PH SIDE 2POS 2MM SMD
1	15	XF2L-0625-1A	OMRONXF2L-0625	OMRON	Connectors - SMD FPC Connectors 0.5mm pitch 6 pos Top contact ZIF Au
1	R57	TSM4YJ203KR05	TSM4YJ203KR05	Vishay Sfernice	20 kOhms
4	S2, S3, S4, S5	SKQMARE010	HDR1X2	ALPS	
1	RESET1	SKRKAEE010	SKRKAEE010	ALPS	SMD Tactile Switches 3.9x2.9x2.0mm 160gf
1	13	292304-2	292304-2	MOLEX	USB Connectors MICRO USB B RECPT BTTM MOUNT ASSY
1	91	J0026D21BNL	J0026D21BNL	Pulse	Ethernet Connector
2	12, 14	5103311-1	HDR2X5	TYCO Electronics/AMP	
	8	142-0701-871	SMB	Emerson / Johnson	RF Connectors PC END BLKHD JCK GLD .062 BOARDS
1	11	TFT	HDR2X17	N/A	
1	U1	STM32F103VET6	LQFP100_L	STMICROELECTRONICS	MCU ARM 512KB FLASH MEM 100-LQFP
1	61	826629-2	HDR1X2	TYCO Electronics/AMP	
1	S1	826629-2	HDR1X2	TYCO Electronics/AMP	
1	110	826629-6	HDR1X6	TYCO Electronics/AMP	
1	U8	RS3-0505D	HDR1X7	RECOM	
1	D10	1N4148W	SOD123	Multicomp	
17	D6, D7, D8, D9, D11, D12, D13, D14, D16, D17, D18, D19, D20, D21, D22, D23, D24	1N4148W	SOD123	Multicomp	
2	U13, U15	ADR433BRZ	SOIC8NA_M	Analog Devices	
1	U14	DS1077Z-66+	SOIC8NA_M	MAXIM INTEGRATED PRODUCTS INC	
2	U12, U16	OP27GSZ	SOIC8NA_M	Analog Devices	
1	U17	OPA627AUE4	soic8na_m	Texas Instruments	
2	a2, a3	BC817-16,215	SOT23-3L	NXP Semiconductors	Bipolar Transistors NPN 45V 500mA
1	Q1	Si2305CDS-T1-GE3	SOT23-3L	Vishay Siliconix	P-Channel 8 V (D-S) MOSFET
1	U3	TC2117-3.3VDBTR	SOT-223-DB3_N	Microchip Technology Inc.	Low Dropout (LDO) Regulators 800mA Fixed Output
1	U4	USBLC6-2P6	SOT-666	STMICROELECTRONICS	IC ESD PROTECTION HS SOT-666
1	60	AD5933YRSZ	TSSOP16_M	Analog Devices	
1	U6	ENC28J60	SSOP28_L	Microchip Technology Inc.	Ethernet Controller
1	D15	LM336-2.5	T0-92	National Semiconductor	
1	U2	LTC4054XES5-4.2	SOT23-5_L	Linear Technology	IC CHARG BATTERY L-ION TSOT23-5
1	US	PCA9543APW	TSSOP14	Texas Instruments	Encoders, Decoders, Multiplexers & Demultiplexers 2Ch I2C-Bus Switch
2	U10, U11	PCA9535PWR	TSSOP24	Texas Instruments	
1		1599ETSBK		Hammond Manufacturing	Enclosures, Boxes & Cases 3.35X6.7 BLACK


4.5 iBioZ circuit diagram and bill of materials

Designator	Description	Footprint	Quantity
C1	Polarized Capacitor (Radial)	1206	1
C2	Polarized Capacitor (Radial)	C1206	1
C3, C4, C5, C6	Capacitor	805	4
C7, C8	Capacitor	1206	2
H1, H2	Header, 2-Pin	HDR1X2	2
In, Out1, Out2	SMB Straight Connector	SMB_V-RJ45	4
J1	Audiosocket	Audiosocket	1
J2, J3	CR1632 Battery Holder	CR1632 holder	2
R1, R2	Resistor	805	2
U1	AD8030	8 pin SOIC_N	1
U2	AD820	8 pin SOIC_N	1