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HIGH DENSITY MICROELECTRODE ARRAYS FOR DETECTION AND CHARACTERIZATION OF RARE CELLS

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Abstract

Much research has been done on biosensors employing microelectrode arrays for biomedical applications. An important application is the detection of circulating tumour cells for cancer diagnostics and treatment. This and many other applications require dealing with a large number of cells, leading to a need for large microelectrode arrays. Fast measurement approaches are required to process these large arrays in the available timeframe. One suitable approach is electrochemical impedance spectroscopy (EIS) if compelling results can be obtained from high frequencies. However, basic design approaches do not scale well with the growing number of electrodes. The increasing need for circuits providing addressing and signal processing leads to mounting parasitic couplings and intensifies the problem of matching between the individual electrodes. While the electrode/electrolyte system is well modelled in the literature, the overall system requires more detailed understanding to improve measurement quality. Moreover, methods need to be developed to improve the specificity of measurement results.

Two previously developed active CMOS biosensor chips were studied for this Thesis. The first of these designs was a basic architecture integrating an array of 96x96 microelectrodes and a tree structure for selecting individual electrodes. The second design featured an array of 104x104 microelectrodes, and improved selection scheme and amplifiers close to the electrodes to improve signal integrity. For comparison, passive biosensor chips made from gold electrodes on a glass substrate were studied. Analytical models were developed to improve understanding of the results, and simulations were done to support the findings. Surface treatments with aptamers were explored to improve the selectivity of the biosensor. A new CMOS biosensor incorporating lessons from the previous designs was developed.

In this Thesis, the design and manufacturing of the used biosensor chips is described with emphasis on design principles and post-processing. Measurement results are presented and compared. Expanded models are derived to give an improved explanation of the observed results. Simulations results are presented that support the new models and indicate possible improvements of the electrode structure. Improved cell capture utilizing aptamers is described, as well as the effects of the aptamer layer on EIS results. Finally, the new biosensor design featuring source measurement units (SMU) for EIS is presented.

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Chapter 1: Introduction

1.1. Motivation

Cancer remains a major cause of death. While new therapy options have improved the chance of survival for many types of cancer, they have also driven up costs of treatment [1]. Hence, it is important to detect the disease, track its progress and decide on the most effective therapy options. It is possible to investigate the properties of the cancer using cells that have detached from the tumour and are circulating in the blood stream. While these circulating tumour cells (CTCs) are not well suited for early detection, they are representative of the properties of the tumour they have originated from. Hence, CTCs are a particularly useful indicator in cases where the disease has already progressed far and there is considerable time pressure to find an effective treatment quickly. Even in later stages of cancer, the detection of CTCs is challenging, though. The required blood sample is easy to obtain, but CTCs are outnumbered in the sample by regular, healthy blood cells by several orders of magnitude. The sample can be filtered to reduce the undesired cells, but this risks losing CTCs and thus distorting the results. Consequently, a considerable number of non-CTCs will pass through the filter, and it is necessary to differentiate between CTCs and other cells for accurate cell counting. An established procedure for CTC enumeration relies on manual labour. In this technique, cells in the enriched sample are stained to improve the contrast, and the sample is then scanned using a microscope. The process requires a trained operator, though, who needs to spend significant time on the counting. The monotonous task is also sensitive to human error. Moreover, the staining step affects the sample and impedes further analysis of the cells.

To avoid the limited contrast from optical measurements, it is possible to scan the sample using Electrochemical Impedance Spectroscopy (EIS). However, single cell resolution is needed for the scan to allow for exact counting and differentiation between CTCs and other cells. In order to facilitate further investigations, it is also highly desirable to capture the cells for detailed measurement. Microelectrode arrays offer a way to achieve these goals, but challenges remain for their implementation. The high electrode density required to achieve single cell resolution demands small structure sizes. A large number of electrodes needs to be addressed, and circuits need to be integrated with the array to facilitate this. Both of these increase the amount of parasitic couplings in the system, which has a significant impact on signal quality. Another major remaining issue is selectivity, as EIS provides insufficient contrast for differentiation between CTCs and other cells of similar size. Surface modification at the electrodes has the potential to improve selectivity, but open questions remain in processing. The impact on EIS measurement and on electrode properties needs to be studied as well. Even design questions on system level remain, as there are several approaches to EIS measurement, and there is a need to

evaluate the options with respect to high density integration. All of these issues present research opportunities in the design of microelectrode arrays. What is more, while the focus in this Thesis is on the detection of CTCs, the underlying challenges are not unique to this application, and the results are applicable to a wide range of biosensors.

1.2. Objectives

This Thesis represents an aspect of the development of an EIS array biosensor for CTC detection and enumeration. The final goal is the detection of CTCs in an enriched sample contaminated with other cells. This requires a high-density microelectrode array with thousands of electrodes. Such an array is feasible if circuitry is integrated with the electrodes. For a practical system in clinical usage, though, the detection has to be reliable and meet severe time limits. The sensor must also be able to detect CTCs with single cell precision in spite of sample contamination with other cells.

It has also already been demonstrated that EIS is capable of the desired cell detection, and the approach can be integrated on chip. However, considerable challenges remain in the design of reliable and selective microelectrode arrays, and a fully operational system is beyond the scope of this Thesis. Besides CTC detection, these challenges also affect similar biosensors with a wide range of applications. Consequently, this Thesis is focused on design issues of active Complementary Metal Oxide Semiconductor (CMOS) microelectrode arrays using EIS as sensing mechanism. In particular, the impact of high-density integration on signal integrity needs to be investigated. Moreover, differentiation between cells using EIS alone is difficult, and techniques are needed to improve the selectivity of the sensor.

1.3. Major Contributions of the Thesis

A considerable amount of work had already been done on the design of biosensors before this Thesis, both in the literature and within the work group. Nonetheless, a number of meaningful contributions are made in this Thesis. In particular, two integrated biosensor chips had previously been designed in the work group in collaboration with external partners, but a large portion of their characterization and evaluation became starting points for this work.

Issues in the design have been identified and studied on the first of these biosensors, featuring a CMOS microelectrode array with a 96×96 electrode field. This design was chosen, as it integrated only the most elementary function, the addressing of individual electrodes. The regular structures helped analysing the results, even though the structure also exhibited considerable parasitic couplings. New analytical models have been developed to improve the understanding of these couplings and their impact on measurement.

The impact was also studied in experiments. A passive chip built on a glass substrate was used as a control, as the isolating base caused less parasitic couplings than the CMOS structures.

Significant differences in behaviour were observed between the two chips, and the new analytical models were used to explain the observations. The new models were also used to identify the responsible structures in the design, and the effects of the couplings were then further investigated in simulations.

Building on lessons from the first biosensor, the second previously designed biosensor was studied with a focus on further integration of the system. In particular, the design integrated amplifier chains on chip. The chip behavior was characterized and design lessons were drawn from the results and the underlying structure.

A third biosensor was developed based on experience from the second design and lessons from parasitic analysis. The key feature of this sensor was a novel measurement topology, performing both sample interrogation and measurement on the same electrode. This topology improves on the locality of the EIS measurement and reduces the active parasitic losses. The design was developed from system level to layout and studied in simulations.

Advanced electrode structures were studied to improve the signal strength of the biosensor. Using suitable multi-electrode setups, the sensing field can be focused and the influence of the electrode/liquid-interface on measurement minimized. In this work, such structures are studied using simulations, and the compatibility with the novel and the conventional EIS measurement topology is evaluated.

To improve the selectivity of the sensors, a surface treatment with an aptamer solution was developed. This treatment was applied to the same biosensors that had been used for parasitic analysis. In this Thesis, it is shown that the surface modification improves the selectivity of the biosensor considerably. The difference in the impact of the treatment on cell attachment is illustrated using two cell lines with different binding behaviour. Using measurement results for varied process parameters, it is also shown that the surface modification need not affect EIS measurement more than exposure to pure water.

1.4. Organization of the Thesis

This Thesis is structured in two parts. To provide the necessary background, chapters 2-4 describe the context of this work. The later chapters then build on this foundation to discuss the work done for this Thesis.

In order to avoid starting with Chapter 2 literally, I need to turn this statement around a bit. The general context of the work is presented in chapter 2. The medical relevance is discussed, pre-processing of the sample is considered and a survey of biosensor applications is given. Chapter 3 is focused on the manufacturing of EIS biosensors, particularly the post-processing. Moreover, the evaluation of the biosensors is also covered. In the course of this description, common structures of passive and CMOS microelectrodes are introduced. Building on this, chapter 4 describes the modelling of these common structures. The theory of the electrode/electrolyte interface is explained. Analytical models of the EIS setup are presented,

from basic equivalent circuits to improved models for the treatment of parasitic couplings. Modelling approaches for EIS simulation are shown, together with results illustrating the effects of parasitic couplings around the electrodes.

The differences between passive and CMOS biosensors are highlighted in chapter 5. In this context, the previously designed biosensors used for this Thesis are described in detail. Measurement results are compared and explained using the improved analytical models introduced in chapter 4. Worst case structures are identified and lessons are derived from the results to design improved active CMOS microelectrode arrays. Finally, system level aspects of microelectrode array design are considered.

Chapter 6 presents methods to affect cell attachment to the electrodes. The focus is on electrode surface modification with aptamers. The preparation of self-assembled monolayers of aptamers is described together with ways to study the impact on electrode performance. The improvement in cell attachment is explained and illustrated with results from cell culture experiments.

Moving from relatively basic structures to more elaborate integrated design, chapter 7 starts with the evaluation of the microelectrode array with integrated amplifier chain and highlights design lessons drawn from the earlier designs. Chapter 8 builds on these lessons to illustrate how changing from a conventional EIS measurement setup to a SMU-based approach reduces the impact of parasitic couplings through improved locality of EIS measurement, and how this reduces the impact of parasitic couplings. The development of a CMOS microelectrode array implementing this approach is presented. Moreover, improvements to the electrode structures that could not be implemented with the design are described.

Chapter 9 analyses the remaining shortcomings of the system. Ideas are presented that could not be investigated in the scope of this Thesis. Finally, the design steps needed to move forward to a complete, integrated system for detection and enumeration of CTCs are illustrated.

Chapter 2: Literature Review

2.1. *Circulating Tumour Cells*

Despite considerable improvements in therapy, cancer remains a major cause of death. To increase the effectiveness, cancer treatment has recently moved towards highly targeted therapy. This directed approach requires detailed knowledge of the tumour, possibly down to the genetic level [2]. To obtain this information, it is necessary to analyse cell samples from the tumour. An attractive way to obtain these samples is given in Circulating Tumour Cells (CTCs) that have detached from the tumour and are circulating freely in the blood stream.

Although they are responsible for forming metastases if they get attached in a different part of the body [3], the diagnostic significance of these cells became clear only as late as 2004 [4, 5]. Nonetheless, CTCs have now become a major indicator for the growth of the tumour [6, 7] and the aggressiveness of the disease [8]. The level of CTCs in the blood is also a predictor for survival rates in cancer patients [9].

CTCs can spread from the main tumour in significant number even early in the disease [10], but they remain necessarily rare. Because they are responsible for the creation of metastases, an accumulation of a sizeable number of CTCs could only happen after a long time of aggressive tumour growth. At the same time, the cells remain rare because very few CTCs do not survive well in circulation [11]. For more practical cases, it has been estimated that even five CTCs make a difference for diagnosis [12]. At the same time, CTCs are vastly outnumbered by similarly sized white blood cells (WBCs) [13]. Consequently, the reliable detection of CTCs in a blood sample is extremely challenging.

2.2. *Sample Enrichment*

A first step to manage the ratio of CTCs to other cells is to filter the sample. This is helpful to remove undesired cells that could be false positives, but it comes at the risk of losing CTCs as well. Because the sample enrichment is so important, a considerable number of techniques have been developed [14, 15].

The most elementary enrichment method for CTCs is centrifugation. As CTCs have a different buoyant density than other cells, they settle in a separate layer during this process [16]. A major advantage of this approach is that only standard laboratory equipment is needed. The centrifugation also allows removal of cell free liquid, making the procedure a good first preparation step. Removing only the CTC layer after centrifugation is not possible reliably, though, so the purification is limited in practice. A better separation is feasible utilizing other mechanical properties of the cells. In the most straightforward approach, Isolation by Size of Epithelial Tumor Cells (ISET), cells can be filtered by size. Such filters have successfully been used to obtain CTCs [17]. Besides the size of cells, their deformability depends on the

cell type, which has also been utilized for filtering [18]. The requirements on the filter membranes are moderate, making mechanical enrichment techniques a straightforward and cost-effective solution. However, the filters can easily be clogged by cells, limiting the sample size. Moreover, as cell properties vary considerably, the filters are likely to either capture too many undesired cells or allow CTCs to pass.

In any case, a hydrodynamic flow is required to move the cells for filtering. Because the interaction of the cells with the hydrodynamic forces depends on its characteristics, a controlled flow can also be used directly for separation of cells. In this approach, there are still losses due to cell variation, but the filter membrane that could be clogged is eliminated [19]. Very good flow control required to achieve good separations, though, because otherwise small variations in cell properties and location can lead to major deviations in behaviour due to turbulent flow.

One method to control the movement of the cells better is Acoustophoresis. In this approach, the forces generated by acoustic waves are used to focus the cells in desired areas of microfluidic channels. This has been demonstrated in a system for CTC separation from similarly sized white blood cells (WBCs) [20]. Similarly, cells can also be focused with Dielectrophoresis (DEP), using dielectric forces in an alternating electric field instead of acoustic forces [21]. In contrast to the speakers required for Acoustophoresis, the electrodes needed for DEP are easily integrated with microfabrication techniques, but the electric field strengths needed for DEP pose a possible danger to the cells. A common weakness of these techniques is that the utilized cell properties are secondary to the cell function. Besides significant variation in the observed characteristics, these characteristics may overlap for different cell types, limiting selectivity.

An improvement in selectivity can be achieved by functionalising surfaces such that they bind to the targeted cells. This has been used to capture CTCs by immobilising antibodies to the Epithelial Cell Adhesion Molecule (EpCAM) expressed by many cancers [22]. Since the expressed surface proteins are determined by the type of cell, the immunocapture method has the potential to be highly specific. The main disadvantage is that a strong bond is formed between the cell and the treated surface that cannot easily be released for cell harvesting. While the bond can be beneficial for detection (see Chapter 6), the immobilisation is undesirable for sample enrichment. A further disadvantage is that only cells touching the surface can be affected.

If small particles are functionalized instead of a bulk surface, these particles can be dispersed in the sample and attach to the targeted cells. By using ferromagnetic particles, the cells can then be affected indirectly without immobilising them. The immunomagnetic approach has been used successfully for CTC capture in the commercial CellSearch system, again targeting the EpCAM marker [23]. While the mobility of the cells is retained using this method, the particles may interact in the magnetic field, leading to the formation of cell clusters. More importantly, the cells are altered significantly by the attached particles. Consequently, it may be more desirable

to target unwanted cells with the immunocapture or immunomagnetic method and thus remove them from the sample, leaving the targeted CTCs behind unchanged. This has been demonstrated in [24]. The negative selection also has the advantage that it can be used on CTCs without the expected surface markers. This is particularly important since CTCs may lose their characteristic surface markers [25]. However, the negative selection requires all unwanted cells to be targeted with suitable antibodies. As a large number of cells has to be removed, it is also likely that CTCs get stuck in the forming cell clusters.

Overall, no enrichment technique can provide the required selectivity and cell retention to allow for reliable detection of CTCs without further differentiation. The optimal approach to sample enrichment consequently depends on the requirements of the following detection step.

2.3. CTC detection and characterization

The developing interest in CTCs has led to a variety of methods for their detection, and a review of these techniques is given in [26]. However, the detection of CTCs is difficult even in enriched samples, because it is to be expected that a significant part of non-CTCs will pass through the filter. This means that any automated detection needs to be able to differentiate CTCs and other cells.

Despite the importance of the cells as diagnostic indicator, only few methods of assessment are cleared for clinical use. At the time of writing, the commercial CellSearch system remains the only test for CTCs that is approved by the Food and Drug Administration (FDA). The system uses blood samples of 7.5 mL and enriches the samples using magnetic particles functionalized with antibodies. For the actual detection, the cells are also stained with multiple fluorescent dyes before an optical scan on a microscope. The cells are then detected through image recognition, although a trained user is required to confirm the results [27, 28]. CTC detection has also been demonstrated using similar techniques utilizing different enrichment methods [29, 30]. Instead

Figure 2.1: Microscope slide grid for cell counting

of the microscope scan of the sample, the selective counting of CTCs has also been demonstrated using a flow cytometer [31].

These methods essentially imitate purely manual enumeration of CTCs, transferring only the final counting step to an image recognition system. Hence, many weaknesses are inherited from the manual process. In manual counting, an enrichment technique is also applied before highlighting the CTCs through a dyeing process. The sample is then scanned using a microscope, and the highlighted CTCs are manually counted. For this purpose, the sample is kept in a microscope slide which is engraved with a grid, as illustrated in Figure 2.1. The visual division of the sample is indispensable to keep track of the parts that have already been inspected. Cells are counted by small squares to keep an overview of counted cells. Cells on borders are assigned to one of the squares they touch according to fixed rules to ensure they are only counted once. The slides are also helpful in ensuring a well-defined sample volume. Nonetheless, the process requires a trained operator, who needs to spend significant time on the counting. It is desirable to reduce the impact of manual labour, and using image recognition or flow cytometers for the counting step is a significant improvement. The required staining steps, however, affect the sample and prevent further analysis and limits the use of these techniques for the characterization of the CTCs found.

A technique better suited for cell characterization is reverse transcriptase–polymerase chain reaction (RT-PCR). Messenger Ribonucleic Acid (mRNA) that is specific to a particular type of CTC is being amplified for use in gene assays [32]. In fluorescence in situ hybridization (FISH), the genetic markers are instead targeted by selectively binding fluorescent molecules. Because of the specificity of these markers, these gene-based approaches are suitable to characterize the cells, but it is necessary to lyse the cells to get access to the genetic material. Because the cells are destroyed, it is not possible to achieve a reliable cell count.

Electrochemical measurement has the potential to achieve both reliable cell counting and characterization. However, whereas CTC detection [33] and cell differentiation [34] have been demonstrated, CTC enumeration and characterization on the same platform is not available yet.

2.4. Structures for Biosensing

The CTC detection using microscopes requires complex sensor equipment, but the arrangement of the sensors is fairly straightforward. In contrast, integrated biosensors attempt to use simple sensors, at the possible cost of complexity in their arrangement. This is particularly true for direct electrochemical measurements, as the sensor elements themselves consist merely of electrodes as a bridge to the wet environment of biosensing. Although the connected measurement circuits can be complex, they are not tied to the particular application except through operating range requirements. The arrangement of the sensor structures, however, must be adapted to the application.

Figure 2.2: Example of a) a flow-through and b) a stationary measurement system

Electrochemical measurements on biological samples can be divided in two categories, passive or active. In the first case, the sensor simply records electrogenic activity from cells. An important example of this are neural probes. In the second case, an electrical signal is applied, and the response is observed. Macroscopic electrodes can be used to analyse tissue *in vivo*. Examples include body fat analysis and tumour detection [35]. For the examination of samples of tissue or blood, smaller electrodes are needed, because the electrodes need to shrink with the subject of measurement. With the advancement of MEMS technology, the fabrication of microelectrodes for this purpose has become feasible.

The arrangement of the electrodes has a large influence on the quality of measurement. The design options for microelectrode setups can be categorized into flow through systems and stationary systems. In the former case, the sample is passing through between the electrodes. The sensor output is then continuously analysed for signals of interest. In the case of stationary measurement, the sample is held in place. The analysis can consequently be either done in parallel, or the zone of analysis must move to scan the sample. In principle, both approaches can be used for the same tasks. This is best understood if stationary systems are considered as flow through systems with a flow rate approaching zero. An example of a flow-through system is illustrated in Figure 2.2a. The sample begins in the reservoir, and is then pumped through the much smaller sensing area. A separate sink reservoir is needed to take in the scanned sample, since returning it to the original sample would lead to mixing. The stationary system, shown in Figure 2.2b, uses only a single reservoir to hold the sample. The sensing area in this example is partitioned into a large number of fields at the bottom.

An important benefit of flow-through systems is the ability to scan the entire sample with a single, high resolution sensor. This is used in optical cell counters. Instead of pumping the sample between electrodes, it is driven through a light barrier. Because only one beam of light is needed, the costs for the sensor remain acceptable even if high quality optics are used. Similarly, in an electrochemical Coulter counter, only two electrodes opposite each other are required, and the sample is pumped through in between the electrodes. The simple structure is well suited for microfabrication, and examples have been published as early as 1999 [36]. The

impedance of this device only changed by 2% when a particle passed the counter. With a width of only 5 μm , the channel was also too narrow to allow cells to pass. Because the underlying technology had reached the necessary maturity around this time and the required structure is straightforward, a number of Coulter counters were presented by other research groups as well around this time. The main focus of the early design was on fabrication and design techniques, rather than application.

Another research focus was on speeding up the measurement. The disadvantage of flow through systems is that the organisms under test only spend a limited time in the sensing area of the setup. Consequently, only relatively fast working measurement principles can be employed. Even though electrochemistry does not present a bottleneck in this respect, practical measurement approaches need to be adapted or become unfeasible due to this requirement. Nonetheless, Fuller et al. demonstrated a particle counter in 2000 using only electrochemical measurement. The design achieved a counting rate of 100 particles/s [37], which means that on average, the particles were in the sensing zone for less than 1/100 s.

For fast flow speeds, which are required for high throughput, additional challenges arise from the flow of the medium. Part of these are microfluidic issues, as the particles or cells in the sample are of similar size to the structures and interact with the flow. Even in the absence of particles, though, the flow of the electrolyte influences the properties of the electrodes [38]. If the flow rate is not steady, this effect leads to fluctuations in the electrode behaviour and noise in the measurement.

While flow through systems can scan a sample sequentially as it passes through the sensor, stationary setups need to hold the entire sample within the sensing area. For this purpose, early sensors of this type used relatively large electrodes, which are useful to provide statistical estimates like the average number of microorganisms per area. An example of a large electrode biosensor is given in [39]. The actual sensing occurs on the surface, though the sensor is meant to be immersed in a solution containing *Escherichia coli* bacteria. In contrast to flow through systems, the sensing time is on the order of minutes. A device using a similar measurement approach on a smaller sample volume was also reported [40]. Although a single measurement was faster in this case, the measurements were repeated over the course of several hours. Since the entire sample is held in place and scanned in parallel, large electrode sensors allow for the longest possible time for examination. However, the inherent averaging over the entire sample provides little information on individual microorganisms in the sample. Nonetheless, these sensors have great potential for cheap and fast analysis of samples where accuracy is not required.

A border case between flow-through and stationary setups is given in systems that catch or extract cells from a stream. Both microfluidic and electrophoretic methods have been investigated for this purpose. The extracted cells are then held in place and analysed as in the

stationary case. For example, one such design features three sites for isolation of cells. Besides a channel providing suction for cell capture, each site includes electrodes for electrochemical measurement. Moreover, an additional channel provides access to each site for injection of drugs [41]. However, although in this case the microfluidic channels are integrated, external pumps are required for operation. Dielectrophoresis (DEP) was also used to extract microparticles from a stream. Instead of individual beads, a large number of targeted particles are drawn onto large electrode pairs. These electrodes are then used for electrochemical measurements [42].

A major drawback of cell extraction from a stream is that significant forces are applied to the cells, which can damage the cells [43]. Another issue that must be considered is that if the cells are not detected beforehand, the extraction needs to be permanently activated until a cell is caught. Moreover, after extraction of the cell, additional time is needed before the extraction is detected. During this time, the cell is still under stress, contributing to the danger of damage.

To increase accuracy of measurement, the sample volume needs to be divided into smaller sensing areas. The division of electrodes into smaller units results in microelectrode arrays. As the area of an individual microelectrode decreases, the possible number of microorganisms in its sensing field decreases. The smaller the area of the microelectrode, the larger is the signal difference for each additional microorganism. In the ideal case, one microelectrode only allows for a single microbe or cell in its sensing area. This allows for a direct, accurate counting of cells or bacteria using microelectrodes.

For tasks that do not require high resolution, lower electrode densities are preferable. For instance, an array reported in 2004 had only 4 by 4 electrodes with a pitch of 250 μm , but it provided an integrated solution for stimulation of electrogenic cells and recording their activity [44]. Single cell resolution is not needed in this case, as the goal is to observe interactions beyond the immediate neighbourhood of cells. A much larger array with 24 by 24 microelectrodes was reported in 2008 for DNA analysis, which does not require high resolution, but rather a large number of sensing sites. Nonetheless, the sensor featured a pitch of only 100 μm [45]. A more advanced version of the chip was also used for neurotransmitter detection, besides DNA analysis [46]. The electrode layout remained essentially unchanged, but the degree of integration on chip was increased, namely with respect to the readout circuitry. Several more examples of microelectrode array applications are covered in the review paper [47]. Building on the presented technologies, the paper makes the case for the promise of microelectrode arrays for the biomedical field. It also shows the versatility of the technology, as the presented arrays are realized in various ways and applied to different tasks.

Microelectrode arrays are mostly interesting for direct electronic sensing, but they also have significant potential with other measurement approaches. A key advantage in that case is that the electrodes can be utilized to drive processes, independent of the actual sensing mechanism. This has been demonstrated using DEP for manipulation of cells [48]. Because the circuitry

needed to facilitate this is minimal, a pitch of 20 μm and a total of 102,400 electrodes is achieved. For sensing purposes, the design integrated photosensors. The advantage of this choice was the low space requirement. However, detection of cells with these sensors has been unreliable, particularly without cell staining. Nonetheless, the design illustrates the feasibility of combining electrophoretic manipulation with a separate sensing mechanism on a microelectrode array.

Combining DEP with electrochemical measurements presents additional challenges, as the requirements on the technology do not match. DEP requires relatively high voltage levels to achieve significant force, which may interfere with, or even destroy, sensitive measurement circuits. If the goal is to use electrochemical sensing, other approaches to capture the cells are therefore of interest, e.g. cell capture using microfluidics on a microelectrode array [49]. The required microfluidic channels were formed by etching holes (3 μm) into the electrodes and back etching of the chip. Nonetheless, it was not feasible to integrate circuitry under the electrodes due to process tolerances. This limited the size of the array to 4 by 4 electrodes. However, the design illustrated the value of cell capture by allowing the detection of signals smaller than 1 mV from electrogenic cells.

Microelectrode arrays can also be combined with sensitive chemical detection methods. This is achieved by coating the electrodes with chemicals that react to the targeted analytes with an ionic or electric signal. Coatings of this kind have already been used in the above-mentioned microelectrode arrays [45, 46] to facilitate the DNA and neurotransmitter detection. A logical extension of this approach is given in Chemical Field Effect Transistor (ChemFET) devices. In these devices, the chemical reaction directly controls the gate of a field effect transistor. The advantage is a very high sensitivity, as the strong signal from the reaction is immediately amplified. For the same reason, it is hard to achieve a gradual increase of the output signal for a given range of input. A more important weakness of ChemFETs however is the need to deposit the receptor chemicals over the gate. Standard CMOS (Complementary metal–oxide–semiconductor) technologies do not offer this option. Nonetheless, for relatively basic sensing applications such as pH measurement, small arrays are possible using dedicated technologies. In 2012, Larramendy et al. presented a measurement system with four ChemFET devices on one chip [50]. Because of the technological limitations, though, little electronics was integrated on the chip besides these devices.

Direct electric measurements, on the other hand, offer good opportunities for integration with existing standard MEMS (Microelectromechanical systems) and CMOS technologies. Additional process steps are needed to encapsulate the chips, but these steps are possible as post-processing. It has to be noted that the electrode surface still has a significant influence on the quality of measurements. However, the sensitivity to process variation is lower than in sensors employing surface chemistry for detection. The impact of surface chemistry on process

sensitivity also needs to be considered when comparing electrochemical measurement approaches.

2.5. EIS Application in Medical Biosensors

The two aspects that are measured by EIS are the properties of the medium between the electrodes and the properties of the interface between the medium and the electrodes. The properties of the interface mostly indicate analytes in the electrolyte. The behaviour of the interface is, however, largely determined by a few dominating factors, and especially by the ion exchange between the surface and the solution and in which may be involved many types of ions from the solution [51]. Therefore, this phenomenon does not provide for selective sensors and, consequently, it is of relatively low importance in medical biosensors. In contrast, the analysis of the medium between the electrodes has a wide variety of meaningful applications.

EIS is a mature measurement approach, and measurement parameters tend to fall into an established range. However, the parameters need to be adapted to the application, and exceptional values can be found in the literature. This is illustrated in Table 2.1. While Jiang and Spencer, and James et al. use conventional signal amplitudes of 50 mV_{p-p} and below, Cho et al. use a much higher amplitude of 500 mV_{p-p}. This is high enough to affect electrode chemistry and is hence usually avoided [51]. The utilized frequency range is also unusually high. Nonetheless, the system achieved valid measurement results.

If the electrodes themselves or their surface-related phenomena are not the target of analysis, it is best if they do not have a big impact on the measurement. This is best achieved by large electrodes, and these are also straightforward to manufacture. A disadvantage is that the resolution is limited by the number of electrodes and, due to their large size, generally poor. Nonetheless, the approach has successfully been used for tumour tissue detection [52]. The change of tissue affects the entire spectrum observed during impedance spectroscopy. However, because in this case the current flows close to the surface, the approach is not useful to detect tumours deeper inside the body.

An extension of EIS tissue analysis that is suitable to address this issue lies in the addition of more electrodes. By surrounding the tissue and measuring the impedances between every electrode pair, the conductivity in the tissue can be mapped. This is called electrical impedance tomography (EIT). Whereas other methods, such as X-ray imaging, have higher diagnostic value, EIT has the advantage that it can be done more frequently without endangering the patient. An EIT system for mammography had a total of 128 electrodes, and was capable of

Journal Article	Signal Amplitude	Frequency Range	Data Points
JIANG and Spencer, 2010 [63]	10 mV _{p-p}	0.1 Hz – 300 kHz	63
JAMES et al., 2008 [57]	50 mV _{p-p}	1 Hz – 100 kHz	26
CHO et al., 2009 [56]	500 mV _{p-p}	40 Hz – 10 MHz	200

Table 2.1: EIS Parameters in Biosensing

locating a 4 mm target in an electrolyte solution [53]. The conductivity of the solution was chosen to match typical breast tissue. A drawback of the design is that it would require the breast to be immersed in a solution matching the tissue of the patient. In clinical use, this renders the measurement more cumbersome and time consuming. More importantly, it keeps the method from being used as a regular screening at local clinics, which would present the greatest impact in early detection. Nonetheless, EIT has huge potential for clinical diagnostics.

The resolution of EIT is tied to the size and pitch of the electrodes used. Consequently, EIT with smaller electrodes has been investigated for microscopic applications, e.g. for culture monitoring. One example used the slime mould *Physarum polycephalum*, but in principle, any cell or bacteria culture could be monitored with the system. The setup is still comparatively large, with a culture chamber diameter of 6 mm and 16 electrodes arranged around the middle of the chamber. As the arrangement is effectively two dimensional, the small number of electrodes is still sufficient to map structures of less than 2 mm diameter with high accuracy [54]. In another example EIT was used in a flow-through system: microelectrodes were arranged around a flow channel of a cytometer instead of a culture chamber. The microelectrodes were only 20 μm long and around 6 μm wide and arranged in two groups of six electrodes around a round channel with 20 μm diameter. This setup allowed to observe the shapes of passing cells in simulations and match them with geometric models [55]. As a side effect, the location of the cell is detected. In conventional Coulter counters, the location of the cell affects the detection, but this can be compensated with the multi-electrode EIT setup.

An alternative approach to gain more information about individual cells is to capture them from the stream and interrogate them with EIS for an extended period of time. Longer examination allows for both higher frequency resolution and a better signal to noise ratio. In [56], the long interrogation was used to differentiate cancer cells from two different cell lines. The cell lines have very different metastatic behaviour, so the diagnostic value of this differentiation is high. 200 data points were taken in EIS measurements for each captured cell, which is only feasible because the cells are captured and held in place. Once they are captured, cells can also be monitored for long periods of time to examine the viability of cells [57]. Trapped cells were monitored for up to two hours using EIS. This kind of monitoring is mostly relevant for observing the effects of drugs. However, long term observation may also have diagnostic uses assessing the health of trapped cells.

In other cases, examination of cell cultures from a sample may be a more appropriate tool for medical diagnostics. An important possibility for fine structured electrodes in culture monitoring is the use of interdigitated electrodes [58]. This configuration, illustrated in Figure 2.3a, is relatively easy to manufacture, but it provides a well-controlled distance between the electrodes, as well as a large surface area. These properties make the design well suited to culture monitoring tasks if a statistical average over the entire population is sufficient for

Figure 2.3: a) Structure of interdigitated electrodes; b) electric field and cell sensing

interpretation. Figures 2.3b and 2.3c illustrate the sensing of cells on the interdigitated electrodes. Due to the proximity of the electrodes, the field remains concentrated close to the surface (Figure 2.3b). Cells or other particles that have settled on the surface interrupt the current flow (Figure 2.3c), increasing the measured impedance. Although the EIS signal provides only limited information on the type of cell or pathogen in a culture, the culture's density and growth rate can be estimated with fair accuracy. A way to improve the selectivity of the sensor is to use electrodes with modified surfaces. This has been used for the detection of human cervical cancer cells with EIS [59].

Electrodes for cell culture monitoring with EIS are comparatively large, as they are meant to observe the entire culture with little effort. If higher resolution is required, the electrodes need to be scaled down, since the size of the electrodes is tied to the resolution of the setup. To enable a precise and accurate cell count, the resolution needs to be on the order of the cell size. The reliability of the cell count improves the trustworthiness of any diagnosis based on it. This is particularly true if the cells are rare. The most common structure for cell counting is the flow cytometer or Coulter counter. The operating principle is that the sample is driven through an opening between two electrodes. If a cell passes through, it displaces the surrounding electrolyte. This reduces the conductivity between the electrodes, and an increase in impedance is detected. Both the opening and the electrodes need to be small to ensure only a single cell is within the sensing area at a given time. Otherwise, multiple cells may be mistaken for a single cell. The disadvantage of the smaller electrodes is that their impedance is increased. To compensate, it is necessary to use higher frequencies, but in this case, the impedance measurement is limited through capacitive coupling across the cell membrane or bypassing the electrode setup.

The electrodes used in Coulter counters are small compared to those for cell culture monitoring, but still large compared to the minimum dimensions of microfabrication techniques. This has allowed many groups to develop MEMS-based Coulter counters. An early example was primarily concerned with the design and fabrication of the setup, but it also demonstrated counting of red blood cells [60]. As the density of red blood cells is a basic indicator for blood quality and health, this has significant value for medical diagnostics. A more sophisticated differentiation with implications for medical diagnostics was achieved in a system that differentiated between red blood cells that were infected with malaria and non-infected cells

[61]. This is possible because the infection changes the properties of the red blood cells significantly, as the malaria-causing protozoans invade and consume the cells. A major advantage of the system is that it does not require expensive instrumentation, but only a MEMS flow cytometer and a basic EIS measurement system based on the chip AD5933.

Coulter counters provide tremendous benefits at low technical effort. However, they provide only a short window of time for analysis as the cell passes by. It is also necessary to avoid clusters of cells entering the analysis window. Because the cells are not uniformly distributed, the cell density has to be kept low. That means that for most of the time, no cell will be in the sensing area. Hence, the time for detection and analysis is reduced further. If the flow speed is not well controlled, the available time may also fluctuate. The scarcity and uncertainty of time reduces the reliability of measurements and the amount of data that can be gained. Whereas detection of cells is fairly straightforward, differentiation is only feasible if the cells differ considerably in their properties.

Stationary setups are superior to flow through designs with respect to time for analysis, because as the cells are settled on the sensor, the time for analysis is only limited by cell degradation. The stationary approach also enables electrode surfaces to be modified for improved selectivity [62]. In order to be able to scan the sample, however, it is now necessary to move the sensing area. This is achieved by splitting the electrode area into an array of microelectrodes. By activating the sensor electrodes in sequence, the active area is moved across the overall sensing area. The array organisation also enables proceeding with a fast but less accurate measurement first. The interrogation effort can then be focussed on the most relevant positions found in the first scan. The sensing resolution is defined by the electrode density, since each electrode provides one data point. If the electrode pitch is on the order of the cell diameter, single cell resolution is possible. The diagnostic capabilities of this approach are highlighted by a microchip for HIV (human immunodeficiency virus) diagnosis realized by Jiang and Spencer [63]. Using antibodies to achieve selectivity, the design achieved accurate counting of CD4+ cells, an important indicator for the progress of the disease, with EIS. However, the chip does not include any circuitry. Hence, electrode selection and EIS measurement are all done externally, which severely limits the scalability of the design due to the large required number of interconnects.

For a passive microelectrode array, routing and connection constraints limit the feasible number of electrodes to a few hundred. This also limits the number of cells that can be detected and analysed on the array. Figure 2.4 shows a 6×6 microelectrode array and a common counter electrode with an EIS measurement system. The counter electrode is driven by a voltage source, and the current into a selected working electrode is measured. The array illustrates the congestion of contacts to the inner electrodes, as almost all paths between the outer electrodes are occupied. A larger array would require either additional layers or a wider electrode pitch. If

Figure 2.4: Passive microelectrode array with cell

this is not possible, the array is limited to 36 microelectrodes. In medical diagnostics, however, large quantities of cells must be handled. Hence, the need arises to integrate circuitry to allow an increased number of microelectrodes. Even for smaller arrays, the integration of circuits for measurement improves the signal quality. However, the additional circuits also introduce new problems, such as noise sources and increased parasitic couplings. The reliance on standard CMOS technologies for the integration of circuits also introduces additional constraints for processing. Nonetheless, the potential for microelectrode arrays using EIS as sensing mechanism in medical diagnostics justifies the effort.

Chapter 3: Fabrication, Packaging and Test of Electrode Arrays

3.1. Fabrication of Microelectrode Arrays

Electrodes represent the central unit of EIS based biosensors, as they form the interface between the electric measurement circuits and the ionic medium of the biological sample. Arrays of microelectrode expand on this approach by offering the capability to perform detailed measurements on sections of a stationary sample. In principle, the same techniques can be used for the fabrication of single electrodes as for electrode arrays, but the complexity of the structures introduces new challenges to the manufacturing process. Microelectrode arrays can be divided into passive arrays and active arrays. Passive microelectrode arrays are defined by their lack of active components, such as transistors or switches, and in practice comprise merely the actual electrodes, connecting wires and contact pads. Active microelectrode arrays, on the other hand, are limited in the number of integrated components only by the size of the available area. Accordingly, the underlying technology of passive microelectrode arrays is considerably simpler than in the case of active arrays.

An example of processing steps for a passive electrode array is illustrated in Figure 3.1 on a single electrode without lead wires. The fabrication begins with a blank substrate. Common

Figure 3.1: Passive microelectrode processing

materials for this purpose include silicon and glass. At first, a series of metal layers is deposited on the substrate, although despite the inner structure, the created stack represents just a single metal layer from the perspective of routing. The lowermost metal serves as adhesion layer, and at least one further metal layer is required to provide a corrosion resistant surface, with gold being a common choice. Once all metal deposits are prepared, a first lithography step is performed to determine the structure of the wires and electrodes. The structuring of the metal layer is then completed with an etching process. After removing the remaining photoresist, one or more isolating layers such as silicon dioxide or silicon nitride are deposited on the wafer. Finally, the isolating layers are structured similar to the metal layer.

Although the fabrication is relatively straightforward, the processing includes many possible sources of manufacturing errors. Residues or irregularities on the surface can affect the adhesion of the metal layer in spite of the dedicated adhesion layer, leading to peeling of metal flakes off the surface. Besides creating an immediate damage to the wafer, the loose flakes of material also pose a contamination risk for processing equipment. A different kind of manufacturing errors may result from flawed etching conditions. If the attack from etching is too weak, structures on the wafer remain that would need to be removed, while excessive etching on the other hand may remove too much material.

This risk also contributes to the minimum structure size, as narrower formations are more likely to be destroyed. The dominant factor for structure is lithography, however. The wavelength of the light used for patterning of the photoresist is of minor importance for electrode arrays, since the structures are comparatively large. Rather, the minimum structure size is limited by the ability to produce a sharp image of the structures in the resist. The major obstacle for achieving this is unevenness of the wafer, as the optical focus is limited to a certain depth. Another important issue is the alignment between the two lithographic steps. As the projected images do not overlap perfectly, an offset between the opening of the isolating layer and the electrode below may exist. A mismatch may expose the lower metal layers to the electrolyte solution, risking damage to the chip and contamination of the sample. The constraints of the lithography also affect the ability to expand the technology with additional metal layers. Though in principle, the process sequence can be repeated to create additional metal layers for routing, the danger of manufacturing errors increases with each additional layer, because the unevenness increases and every new layer poses an additional risk for misalignment.

In the case of active microelectrode arrays, it is most efficient to sidestep most of these issues by building on an established CMOS technology provided by a foundry. The main disadvantage of relying on the external fabrication is that control over process parameters is exclusively with the foundry. Thus, the technology is not well adapted to the needs of biosensors. Consequently, it can in principle be useful to develop a dedicated process for the production of microelectrode arrays. This is only feasible for low grade technologies with large

structure sizes, however, since otherwise costs are prohibitive. Moreover, whereas it may be possible to improve individual features of the technology, such as uniformity or biocompatibility, this is likely to come at the cost of overall performance. Thus, it is generally preferable to create active CMOS microelectrodes based on fully processed wafers from a foundry. Since biocompatible surfaces are not required for most circuits, however, suitable surface finishes are usually not available.

It is nonetheless possible to use the microelectrodes as they are delivered from the factory, but the exposed contacts are likely to be attacked by the electrolyte solution and in turn release toxic substances into the solution, affecting the sample. The attacked surface also does not provide a stable measurement response, so reliable measurements are not possible. Hence, compatibility between the technology and the samples is a major concern for high quality biosensors. This is particularly true for active CMOS microelectrode arrays, because biocompatibility is not relevant for standard MEMS and CMOS technology. Hence, the materials used in manufacturing can poison the sample if they leak. Conversely, biological samples contain chemicals detrimental to the electronics. A common problem is that nutrient solutions are highly corrosive to metal wires. In the process, they also dissolve poisonous elements from the circuits. To protect both sample and electronics, the sensor has to be encapsulated with inert materials. Material stacks and process parameters need to be adapted to the application to ensure good adhesion and prevent diffusion of less stable materials to the surface. A possible material stack for a protruding EIS electrode is shown in Figure 3.2. Such an electrode is formed if the isolating layers are deposited and patterned on the substrate before the additional metal layers are added. The chip at the bottom is using SiO_2 as isolator material and Al for the conductors. To seal this chip, a Si_3N_4 layer is used to cover and seal the isolation. Au is used as electrode material, with Ti serving as diffusion block and adhesion promoter.

Post-processing thus essentially follows the same process steps as the fabrication of passive microelectrode arrays. The isolating layer covering the metal may be omitted, however, since all routing wires are already integrated in the CMOS technology and are hence protected. The drawback of this approach is that the adhesion layer of the metal is exposed to electrolyte solution at the sides. Moreover, the oxide forming the higher isolating layers of the CMOS

Figure 3.2: Possible material stack for a protruding electrode used for on-chip biosensing (not to scale)

technology may be of low quality and porous, since it is meant to be covered in a package. Hence, if the post-processing technology allows the deposit of high quality isolating material, the additional processing steps are usually worthwhile. A general problem for post-processing is that the state of the wafer surface as it is delivered from the foundry is not well defined. The metal contacts, in particular, are bound to be covered in an oxide layer, but the thickness and density of the layer may vary with storage time and transport conditions. Nonetheless, this layer needs to be removed to allow good contact with deposited metal layers used to form electrodes. What is more, the surface of the wafer is uneven, impeding mask alignment and patterning of the photoresist due to distortions and lack of focus in optical flow.

A useful post-processing step that can be done without lithography is the deposition of self-aligned monolayers. As discussed in more detail in Chapter 6, these layers can bind selectively on metal or isolator and offer considerable promise in improving selective cell capture. In principle, it is possible to avoid the need for lithography in post-processing entirely. As metal contacts are already present, they can be used to seed autocatalytic growth of the electrodes. By avoiding the need for lithography, this method eliminates the danger of alignment errors, and a perfect seal of the lower metal layers can be achieved. However, the process is hard to control because of its autonomous nature, the selection of materials is limited by their chemical properties and the deposited materials are not pure. Consequently, the technique may not be suitable for the fabrication of biocompatible electrodes in the near future.

Additional challenges for manufacturing may arise from specific needs of the application. In particular, neural implants must be fabricated to withstand the biological environment for years. Moreover, the neural interface benefits considerably from a porous substrate by inhibiting a rejection from the body [64, 65], which contradicts the need to seal the chip from the environment. Even for established techniques, every step of processing and post-processing is sensitive to processing parameters. Because not all of these parameters are easily controlled, the output may vary and the equipment needs to be monitored and adjusted to ensure reproducible results. In the case of CMOS based technology, a considerable part of the process control is already settled by the providing foundry, though the products can still vary considerably. For microelectrode array based sensors, however, the most critical variation is in the characteristics of the electrodes, since these characteristics determine the actual measurement signal. As the electrode properties are determined primarily by post-processing, this aspect of manufacture deserves particular attention. In the case of biosensors, new extensions to post-processing such as surface functionalization, considered in more detail in chapter 5, also offer considerable opportunities to improve the measurement response. This also adds to the importance of passive microelectrode arrays, as the technology offers a less complex background for the development of the same processing steps.

3.2. Packaging of Microelectrode Arrays

As the sensitive biosensor can easily be contaminated by the environment, it needs to be packaged for protection. At the same time, the package has to facilitate the connections with the supporting circuits and the insertion of the sample. Furthermore, it may be necessary to provide access to parts of the sensor for optical inspection. Consequently, the different requirements need to be balanced against each other. Especially the contact with the wet and potentially corrosive environment of electrolytes needed for biosensing applications is potentially at odds with the requirements for electronics. The packaging also has more mundane functions, however, as it needs to translate the bond pads available on the microelectrode array to contacts that can be reliably attached to from a socket.

This is especially important because the biosensor is contaminated by the sample that is being analysed, and hence needs to be exchanged after every test. First and foremost, this puts substantial requirements on the socket, but for these demands to be fulfilled, the sensor needs to provide suitable contacts as well. In particular, the contacts must allow for robust counterparts, which limits the achievable contact density. This may pose a particular challenge for passive electrode arrays, as the number of contacts scales directly with the number of electrodes. The combination of bond pads and electrodes also presents a potential difficulty for bonding, since they are located on the same side of the chip, unless they can be routed through the chip with through-silicon vias (TSV). The electrodes need to be exposed to the sample, while the bond pads need to be shielded from the environment. Due to the delicacy of the bond wires these are usually covered with a polymer for protection, but this polymer needs to be kept away from the electrodes. To facilitate this, the bond pads need to be placed with sufficient distance from the electrodes, raising the area requirements of the chip. The space can be filled with circuits in active CMOS microelectrode arrays, but there may not be enough need for such circuits to fill the space.

The polymer used to protect the bond wires is usually chosen for its fluid flow to ensure a reliable fill between the wires. Consequently, the material must be kept from the electrode field and is not suitable to form a well-defined microfluidic chamber on top of the electrodes. Besides serving as a barrier between the liquid sample environment and the dry environment of the electronics, the chamber also needs to facilitate a consistent fill when the sample is inserted. Consequently, the flow of the liquid into the chamber has to be considered, and corners that could hold bubbles of air need to be avoided, because the air pressure prevents the liquid from entering the corner. The chamber design also needs to take capillary forces into account, as the fluid may be drawn in counterintuitive directions. The capillary forces may, however, also be used to help fill the chamber, as the forces pull the liquid in to fill the space. The drawback to this approach is that the forces attracting the liquid also make it harder to remove liquid from

the chamber or exchange it. Besides the geometry of the chamber, the strength of the capillary forces depends also on the surface properties. To ensure a smooth fill of the chamber, hydrophilic surfaces need to be used, whereas hydrophobic surfaces are capable of preventing the sample from entering a coated area unless increased pressure is applied to the liquid.

If such pressure is to be used to help with filling or cleaning of the chamber, the microfluidic system needs to be designed to withstand this pressure. Otherwise, the risk of electrolyte solution leaking into the protected electronic environment exists. Because the microfluidic chamber is composed at least of two components, namely the chip and a cap, there is at least one weak spot at the interface. In practice, the walls of the chamber need to be created from a material that can adapt to the surface structure, whereas the ceiling of the chamber needs to be solid to prevent caving in. A useful material for the formation of the chamber walls is epoxy, as it can be deposited in the liquid state and then cured. The material is also suitable to glue the solid ceiling to the chamber. A wide variety of epoxy combinations are available, allowing to select a good match for many fabrication requirements. In particular, biocompatible compositions exist with suitable flow characteristics to form walls capable of holding their shape until curing is completed. An alternative to epoxy is polydimethylsiloxane (PDMS), which is also available as biocompatible material and largely inert. Moreover, the material is photopatternable, which has been used successfully for the manufacture of microfluidic channels [66, 67]. However, a separate step may be required to bond the chamber ceiling.

If the design of the microelectrode array requires a common counter electrode, a conductive material is needed to form the ceiling of the chamber. Since optical inspection of the surface is required to assess the state inside the chamber independent of the sensor output, the ceiling also needs to be transparent. The material of choice for transparent conductors is indium tin oxide (ITO), though due to its cost, it is only used as thin film on a less expensive substrate such as glass. Because the conductive side of the ceiling piece must face the electrode array, forming a contact is not straightforward. A possible solution is to use a conductive adhesive that is cured together with the epoxy used to glue the ceiling on the chip. To contact the adhesive, it is then sufficient to provide a connection on the chip surface with sufficient distance to the electrode array, and then bond to this line. Because this connection carries the signal to the counter electrode, it is imperative that the line is isolated from other conductors.

The reservoir chamber must have a well-defined volume to ensure the size of the sample under investigation is fixed. In order for the dimensions of the chamber to be constant, the structure must be rigid. The microfluidic inlet and outlet need to be as small as possible to minimize the impact on measurement. Under the conditions of development, these requirements can be softened, and inconveniences in accessing the microfluidic chamber are uncritical. In practical use, however, handling of the sensors needs to be straightforward to avoid mistakes, and measurement must be reliable and repeatable. Consequently, the microfluidic inlet and

outlet need to be designed to facilitate cleaning and filling of the reservoir without affecting measurement. Because any contact of the sample with the substrate poses the risk of creating undesired connections, the microfluidic channels need to be created in higher layers.

Building the channels with epoxy walls and a glass ceiling similar to the reservoir chamber is a possibility, but the process would require additional curing steps, which may be detrimental to other structures on the chip. Moreover, the process is not cost-effective, as suitable materials are costly and the precise placement of the relatively high epoxy walls is time consuming. Because cells need to be able to move freely through the channels, the channels need to exceed the cell size significantly in every dimension. A more efficient approach is to manufacture the microfluidic channels separately and glue them onto the chip. For prototyping and small series production, the channels can then be manufactured by laser cutting the pathways from plastic sheets before gluing the sheets together to form three dimensional structures. For larger scale production, more scalable fabrication techniques such as casting are needed to create the individual layers. Photopatterned PDMS may also serve as a technique for volume production, but it is less suitable for higher layers because the required lithography may affect lower layers. In any case, the assembly of multiple layers remains necessary, introducing a structural weakness at the seam. These weaknesses need to be minimized, as any leakage would invalidate the sample and potentially damage the system. Another possible leakage point is at the overall system inlet, where the sample is injected into the system. To avoid spillage, a tight coupling between the sample container and the sensor package needs to be guaranteed. This necessitates a relatively large interface to avert the risks of missing or damaging the inlet. The volume of the microfluidic channels must be minimized, however, since any part of the sample that cannot reach the reservoir chamber is not subject to measurement. From a material perspective, the higher layers of microfluidics need to have high elasticity to resist damage from handling, especially pressure and strain during sample injection. Similar to the ceiling of the reservoir chamber, the material used to form microfluidic channels should be transparent to allow optical inspection, though the main reason in this case is the need to detect leakage.

Polymers are a good choice to fulfil these requirements, but they are problematic in terms of cleaning steps on the packaged sensor. To remove organic residues from the microelectrode array, aggressive organic solvents may be necessary, but these solvents may also attack the polymer structures of the microfluidic channels. Unfortunately, organic residues are frequently introduced in the manufacturing process. After wafer processing or post-processing, mask residues may remain on the electrodes, although these can be removed before packaging. The packaging process itself may contribute to the contamination, however, as glue and solvent may be trapped in the microfluidic pathways. Moreover, organic materials used for packaging such as the epoxy used to create the chamber walls may trap gaseous reaction products during curing. Outgassing compounds may then be adsorbed at the electrodes. Because these residues are only

released during or after packaging, additional cleaning steps are needed to remove them, and the materials used in packaging need to be able to withstand the solvents used for cleaning.

The materials used for packaging also need to tolerate any other processing step after their application, and they need to be biocompatible unless it is guaranteed that neither the substance itself or any released compounds get into contact with the samples. Moreover, the materials need to match other parts they are connected to in order to create stable bonds and to avoid destructive chemical reactions. The packaging needs to be sufficiently stable to protect the sensor from damages during handling, but they must not create mechanical strain in the sensitive sensor. Rather, the package needs to absorb strain from the socket it is placed into and protect the sensor from being affected. The packaging of biosensors presents many challenges, and care needs to be taken to avoid detrimental effects on sensor performance.

3.3. Testing of Microelectrode Arrays

Biosensors need to undergo testing at several stages during development and manufacture. Analyses in the development phase are merely concerned with ensuring the full functionality of the design, whereas quality control is the objective in production. Besides avoiding the waste of costly processing steps on defective chips, this also helps to pinpoint the source of damages and design errors. Production tests are harder to design, as only few samples are available for destructive tests and evaluation has to be done under considerable time pressure. Tests during development, on the other hand, are more flexible, but the interpretation of results is more difficult and time consuming. In both cases, it is vital to consider the need for tests during design in order to include required test structures and ensure access to the relevant signals.

Established standard CMOS technologies are undergoing inspections during manufacture, but functional tests can only be performed after wafer processing is completed, because the functionality relies on a completed chip. Moreover, probes are required to contact the wafers for electrical testing. If the tips of these tools are harder than the electrode surface, they are prone to damage the electrodes. The gold surfaces created in post-processing are relatively soft, while probe tips require a certain hardness to avoid excessive wear. Functionalized surfaces are also not suitable for test probes, as the thin surface layers are easily damaged. Accordingly, electrical tests can only be done on dedicated test structures, and optical inspections are used for intermediate examinations. In any case, an interruption of the manufacturing process is undesirable, and wafers are processed completely before testing, unless an alert from the processing equipment signals the need for additional quality tests.

If post-processing is required, damages from probes for electrical testing may pose a problem for lithography due to the uneven surface. However, the state of the surface before post-processing is important for process parameters. Hence, it may be necessary to accept the issues caused by the damaged surface or sacrifice a wafer from the same batch to assess the surface

quality. The results of post-processing also need to be evaluated to ensure mask alignment and surface properties are within specifications. Optical inspections are particularly important for the electrode array, since the electrodes will be exposed to the electrolyte solution, and even miniscule cavities can open the surface to disastrous corrosive attack. After packaging, electrical tests are easier, because the package can be used in test sockets to ensure good connectivity. The provided seal of the chips also allows for transport of the sensors. This is necessary, as wet tests are required for biosensors that cannot be performed in the same clean rooms as manufacture, because the required electrolyte solution is incompatible with CMOS technology. The wet tests are crucial, however, as the electrochemical properties of the electrodes have a substantial influence on measurement. The exposure to electrolyte solution includes a risk of contamination, however, so these investigations have to be considered destructive tests and can only be performed on samples.

In spite of being limited to samples taken from the manufactured batch, destructive tests serve important purposes in development and quality control. A special case of destructive measurements are long term tests. These tests are of particular significance for sensors, because they are needed to evaluate the degradation of the sensing function over long periods of storage or use. For microelectrode arrays, aging of the electrode surface can occur within a relatively short timeframe on the order of hours, as the surface chemistry is affected by the environment. Once filled with electrolyte solution, the characteristics may change even within a single measurement, as ions from the solution diffuse into the electrode and alter the electrochemical properties. These effects need to be investigated to compensate for the variation in measurement results. Ideally, however, the drift is already minimized during manufacturing. A common technique for circuit fabrication to reduce later changes to the characteristics is the so-called burn-in. By accelerating the aging process through heat treatments and other increases of the environmental load, the major shifts in behaviour are settled during production. Similarly, it is possible to reduce the drift of the electrode surface chemistry in biosensors by exposing the surface to a solution that is chemically similar to the samples the sensor is meant to process. The electrode properties are degraded in this process, however, and measurements are nonetheless necessary to assess the surface properties.

For most of the development phase, the drift in electrode properties is a minor problem, since measurement results may vary more severely between experiments. Because of this variability, observations made with the new setup cannot be considered reliably without substantiating the results with established measurement approaches. In the case of EIS based biosensors targeting cells, this verification has to be done through optical inspections. For this purpose, the cells on the array are detected manually, and the EIS results obtained from the corresponding electrodes are compared with results from electrodes that are not covered by a cell. Detection of cells using EIS is not straightforward on an electrode array, because both the

base response of the electrodes and the change due to a cell present may vary. Whereas uniformity of electrode responses can be improved through enhanced processing parameters, the variation in cell behaviour is inherent to biological systems. In practical use, this is handled by the definition of a decision threshold, above which the cells are detected. For development tests, however, a model with better controlled properties may be needed. A possible choice for this purpose are microbeads. These approximate cells as isolating spheres of a diameter similar to the cells under investigation. As shape and size are better controlled than in cells and the isolation persists at higher frequencies, the microbeads are in principle easier to detect using EIS. However, as the shape is fixed, the microbeads do not conform to the surface and hence do not produce a good seal on the electrodes. Consequently, whereas microbeads may be easier to detect in Coulter-counter setups, detection on electrode arrays is difficult. Because the final objective of the sensor remains cell detection, the detection of microbeads is in any case only an intermediate step, though it may be helpful for evaluation.

Before cells can be reliably detected with the biosensor, the microelectrode array needs to operate consistently by itself, and quality control represents a major aspect of ensuring this functionality. As the importance of quality control is inherent to any sensitive equipment, extensive procedures are already in place for established CMOS technology. For post-processing or the manufacture of passive microelectrode arrays, however, specific tests need to be developed. The interaction of the electric components with the wet environment of biosensors sets high standards for the interface and packaging, and the test procedures need to ensure that these standards are met. This can only be achieved if testing is considered at all stages during design.

3.4. Summary and Conclusions

The technology used for the manufacture of microelectrode arrays has a considerable impact on the design of microelectrode arrays. Besides putting constraints on the available feature size, the production process is a crucial factor for the accuracy and reliability of sensors. In their most basic form, passive electrodes and electrode arrays can be created as a single metal layer on an isolating substrate. Whereas this process is straightforward, it is not free of pitfalls, and processing parameters need to be tightly controlled. More complex microelectrode arrays can be built utilizing highly developed CMOS technologies provided by silicon foundries. As biosensors have unique requirements, however, the processed wafers still need to be post-processed using fabrication steps very similar to passive electrode fabrication. Although advanced processing techniques may offer new opportunities for manufacturing, the production of passive electrodes therefore remains a key capability for the development of microelectrode arrays.

Another significant aspect of fabrication is packaging, as microelectrode arrays cannot operate without supporting circuitry. Packaging of sensors is challenging, as any mechanical tensions can distort the sensor response. In biosensors, additional difficulties arise from contact with a wet, corrosive environment. Any leakage in the microfluidic channels or the reservoir chamber on top of the electrodes results in a waste of the tested sample and can have devastating consequences for the electronics. The sample may also be lost to the volume of the microfluidic inlets instead of reaching the measurement chamber, and errors in manufacturing or in the choice of materials can prevent the sample from filling the chamber properly. These effects lead to the risk of measuring only a fraction of the sample, possibly losing crucial, rare cells. Common packaging issues such as limitation of mechanical stress remain important for biosensors as well, particularly if sensitive measurement circuits are integrated. The problems are complicated further, however, because the materials and processes used for packaging need to be compatible both with the electronic and the biological domains. Moreover, as biosensors are contaminated by the investigated samples, the package needs to be exchangeable. Consequently, packaging of biosensors remains a challenging topic, and developed procedures are not easily transferable to different sensor designs.

Even for established designs, tests need to be performed at several points in development and production to detect manufacturing errors in the sensor. To facilitate these tests, it is important to consider verification during design. Since quality management is a necessary step for all CMOS chips, functional tests after processing are standard procedure. For biosensors, additional tests are required, however, and the electrodes cannot be contacted directly without the risk of damaging the surface. In particular, the need for wet tests to ensure satisfactory electrode properties arises. Thus, in the test of biosensors, just as in manufacture, many advantages can be gained from building on the foundations of proven CMOS technology, but the contact with biological samples presents important, new challenges, which need to be investigated.

Chapter 4: Theory and Simulation of Microelectrode Arrays

4.1. The Electrode/Electrolyte Interface

An important aspect of EIS measurement on biosensors is the impedance of the interface between the metallic electrodes and the liquid electrolyte the cells are suspended in. Consequently, models are needed to describe the behaviour of the electrode/electrolyte interface. In practice, the value lies in the interpretation of results, rather than prediction, since the parameters used in the models may be difficult to obtain. The behaviour of the interface is determined by electrochemical reactions at the surface. In these reactions, an equilibrium state is formed between ions bound to the surface, diffusion leading towards a homogenous concentration and an electric field driving an opposing current. For a single type of ion, the process is similar to the movement of electrons and holes in the formation of a diode junction. As there are multiple types of ions involved, and each may be positively or negatively charged, the interface exhibits a characteristic similar to several diodes connected anti-parallel or in parallel. For purposes of EIS, it is sufficient to consider the small signal behaviour of the interface as a result of perturbations of the equilibrium state. The derivation of this small signal behaviour is well established in the literature, and only a summary is given here, based on [51].

Whereas the surface chemistry underlying the ion exchange is highly complex, the relatively simple equivalent circuit models presented in Figure 4.1 is sufficient to describe the behaviour well. In the model in Figure 4.1a, consists merely of a parallel circuit between resistor R_t and capacitor C_l . These elements represent the main coupling mechanisms of the interface. Whereas the resistor represents ions passing directly from the electrode into the electrolyte, the capacitor represents ions being bound or released by the space charge region near the surface. At low frequencies, the capacitive component is negligible, and the observed impedance is given by R_t , whereas for frequencies larger than the cut-off frequency of $(2\pi C_l R_t)^{-1}$, the impedance falls by 20 dB/decade as the capacitance becomes more conductive.

The two-component model reflects the general behaviour of the electrode/electrolyte interface well, and it provides a good representation of the frequency response of microelectrodes. However, a corrective term is needed for high currents to account for the

Figure 4.1: Equivalent circuits of the electrode/electrolyte interface: a) without Warburg impedance; b) with Warburg impedance

limited flow of ions to the electrode. This term is represented by the Warburg impedance, often denoted as a parallel circuit of a resistor R_w and a capacitor C_w in series to R_t as in Figure 4.1b. The capacitive path through C_l is not affected, since the equilibrium layer fluctuation cannot be depleted of charges without violating the small signal assumption. The impact of the Warburg impedance on the absolute impedance is often limited and shows merely in a slower decrease of impedance with frequency. However, the additional term introduces a phase shift to the resistive path, and accordingly the phase of the observed impedance is affected.

In order to estimate the parameters of the components, it is helpful to consider the underlying processes more closely. The interface capacitance C_l is formed by the arrangement of ions in the space charge region. A first layer of charges, the inner Helmholtz plane (IHP), is formed as the ions are bound directly to the surface by adsorption or surface chemistry. Together with free charges in the electrode, this ion layer attracts opposite charges from the electrolyte. These ions are separated from the surface, however, by solvent molecules that have oriented themselves in the electrical field, and an outer layer is formed. Since the second layer is held in place by an electric field from the surface and the ions in the IHP, it fluctuates with the charge on the surface, and a capacitive coupling is generated. Due to the two layers of ions, the resulting capacitance is called a double layer capacitance.

Whereas the capacitance can be largely understood by physical processes, the resistive current transfer from the electrode to the electrolyte relies heavily on chemical interactions. In order to translate the electron current in the metal electrode to an ion current in the electrolyte it is necessary to bind ions to the surface or release them into the solution. If the applied voltage is sufficient to initiate electrochemical reaction, this occurs in the form of electrolysis, but for the small voltages used in EIS, the charges have to be transferred to the ionic compounds in the solution through reactions occurring as part of the electrochemical equilibrium at the interface between electrode and electrolyte. The charges then need to be transported through the electric field of the space charge region between the double layer, and the energy required to achieve this gives rise to the charge transfer resistance R_t . Depending on the electrolyte used, R_t can be large enough for microelectrodes for the total current to be dominated by the capacitive path through C_l . The presence of the transfer resistance is then only recognizable in the phase of the observed impedance.

The Warburg impedance is related to surface chemistry and the charge transfer between electrode and electrolyte as well. Instead of the steady state power required to drive charges across the double layer, however, it represents the load due to insufficient reactants at the surface layer. The reactants are needed for the charge transfer to work, but the concentration of reactants in the electrolyte is limited, and fresh reactants need to diffuse to the surface for the next charge transfer to occur. As the diffusion is related to, but not directly driven by the electric field and the consequential fluctuation of the ion concentration near the surface, it exhibits a behaviour

between a resistor and a capacitor. Hence, the Warburg impedance is often represented through a combination of resistor and capacitor. This is not accurate, though, as the Warburg impedance acts as a constant phase element (CPE) with a phase of -45° and an amplitude which is decreasing with the square root of the frequency. Because of these characteristics, the Warburg element results in a deviation of the observed impedance that is most recognizable in the recorded phase.

For low frequencies, the interface characteristics dominate the EIS results. However, the circuit is closed only through the electrolyte and the counter-electrode. The counter-electrode is usually considerably larger and can be neglected. Conversely, the impedance of the electrolyte is relevant, since the impedance of the interface decreases with frequency. As the current spreads from the microelectrode, the impedance in the electrolyte is dominated by the resistance to the high current density near the interface, and the impedance depends heavily on the geometry of the electrode. Due to this close connection, the spreading resistance can be considered part of the electrode impedance. The spreading resistance R_c of a circular electrode of radius r_{el} in a medium with resistivity ρ can be approximated as:

$$R_c = \frac{\rho}{4r_{el}} \quad (4.1).$$

In contrast, the spreading resistance R_{sq} of a square electrode with side length l_{el} in the same medium is approximately:

$$R_{sq} = \frac{\ln(4)\rho}{\pi l_{el}} \quad (4.2).$$

Consequently, to achieve the same spreading resistance, the relation

$$\frac{l_{el}}{r_{el}} = \frac{\ln(256)}{\pi} \quad (4.3)$$

must hold. However, the relation

$$\frac{l_{el}}{r_{el}} = \sqrt{\pi} \quad (4.4)$$

must also hold to give the same electrode area. Because the transfer resistance and interface capacitance depend on the area, a square electrode and a circular electrode cannot have the same interface characteristics and the same spreading resistance at the same time.

Although the structure of the equivalent circuit for the electrode/electrolyte interface is established and the underlying physics are well understood, the parameters describing their properties are frequently unavailable. As the parameters depend on the electrochemical reactions at the surface, properties of the involved materials and their interactions are needed to derive reliable estimates. However, as impurities can affect the reactions considerably, only approximate values for some combinations of materials are available in the literature. Moreover, the reactions depend on the operating point and on environmental conditions, particularly

temperature. Consequently, instead of calculating an expected behaviour of the electrode, the models are best used to calculate the underlying parameters.

In any case, the equivalent circuit model is valid only for frequencies the solvated ions are able to follow. At frequencies above 1 GHz, the ions remain essentially immobile in the alternating electric field, and the interface appears essentially transparent. With higher frequencies, however, other relaxation processes such as the orientation of dipoles in the field become relevant, and these are not reflected in the model. The interface model is also based on infinitely wide electrodes, and fringe effects are not considered. Whereas this is a fair assumption due to the thin double layer, the influence grows for smaller electrodes. In particular, capacitive coupling from the electrode can reach the electrolyte surrounding the electrode, effectively enlarging the electrode area. The double layer extends beyond the limits of the electrode as well, though the properties change due to the different material combination. Fortunately, the equivalent circuit model suffices to represent the behaviour of electrodes in current microelectrode arrays, but future developments are likely to require improved models.

4.2. Analytic Modelling of Electrode Arrays in EIS Biosensors

Whereas the behaviour of individual electrodes is well-established and can be described with few components, the model does not take into account effects of the environment. In biosensors based on passive microelectrode arrays, the behaviour of individual electrodes is largely preserved, but neighbouring electrodes are capacitively coupled and connected electrodes may serve as additional sink or drain for the current. Sensors based on active CMOS microelectrode arrays add further to the complexity, as many electrodes may be connected at a time and circuits interact with the electrodes. The interface model is nonetheless important, because it reflects the behaviour in the immediate vicinity of the electrode. In the case of biosensors, however, the interface impedance itself is not relevant unless the characteristics are changed through biochemical indicators. The spreading resistance, in contrast, is affected by changes in the electrolyte composition, cells blocking the current, and distortions of the current flow. Because of these influences, the analytical solutions for the spreading resistance do not reflect the complexity of microelectrode arrays well, and the known equations can only serve as estimates.

Even for single electrodes, the model may not be sufficient, as it does not account for cells on the electrode. The simplest model for the impact of the cell is illustrated in Figure 4.2a. In this model, the cell is assumed to be an ideal isolator and forcing all current around it. Hence, current faces a resistance R_{s1} before encountering the cell, then a further resistance R_b representing the effort to bypass the cell, and finally a resistance R_{s2} between the cell and the counter-electrode. In practice, R_{s1} and R_{s2} are combined into a single resistance R_s , as together they represent the spreading resistance without cell. The assumption of the cell as ideal isolator

Figure 4.2: Equivalent circuits for individual cells in the electrolyte: a) cell as ideal isolator; b) with capacitive coupling across the cell wall; c) with complex couplings to bypassing current

is valid for low frequencies, but signals at higher frequencies can couple capacitively across the thin cell membrane and penetrate the cell, represented through membrane capacitances C_{m1} and C_{m2} in Figure 4.2b. As interior of the cell has a limited conductivity that may be higher or lower than the electrolyte, it is represented through resistor R_s . This model is most useful for analysis and planning as it represents the fact that the cell becomes harder to detect with higher frequencies. Consequently, there is a maximum frequency above which detection is not feasible, and this limits the required bandwidth for EIS measurement.

The characteristics of the cell are not fully covered by the model, however, as the current may also partially bypass the cell and capacitive coupling occurs over the entire cell membrane. These interactions are partially modelled in Figure 4.2c. For this purpose, the bypass resistor is split into the components R_{b1} and R_{b2} , and an additional capacitance C_{mi} couples to the cell. The interior is now modelled by the triangle of resistors R_{c1} , R_{c2} and R_{cp} . Though the behaviour of the cell can be modelled more closely in this manner, in practice it is more useful to assess the impact of the cell in simulations. Moreover, variations in cell geometry and composition outweigh the errors in modelling. The variations also mean parameters can only be estimated or fitted to data, and values are rarely available in the literature. Furthermore, even if measurement results are available, fitting data to the results is not always feasible, as the computation may be unstable. Hence, the model in Figure 4.2b remains preferable in most cases.

Another aspect of cell modelling is the impact of cells covering the electrodes directly. An equivalent circuit modelling a partially electrode is given in Figure 4.3. This circuit has been used to describe a comparatively large electrode that offers enough space for several cells to settle on [68], but it is also valid in principle for microelectrodes with dimensions on the order of single cells. The total admittance of the electrode/electrolyte interface Y_i is divided into a fraction α covered by cells and the remaining fraction $1 - \alpha$ that is exposed. Whereas the bare

fraction of the interface connects directly to the spreading resistance R_s , the covered fraction first has to overcome the impedance of the cells, denoted through the admittance Y_c in the figure.

The influence of cells on impedance is desired in biosensors, because it creates a signal that can be detected by the sensor. In contrast, changes to the measured impedance due to other structures of the sensor are undesirable, as they reduce the signal or raise the noise level. One of the most common detrimental effects in EIS based sensors is capacitive coupling between the wires leading to

the electrodes. This coupling can be modelled through a single capacitance in parallel to the electrode setup and leads to additional current reaching the instrumentation. As this current bypasses the actual sensing structure, the effective signal is damped. A constructive solution to this issue is to introduce shielding layers between the relevant leads, but this is not always possible. If the setup can be decoupled close to the electrodes, however, it is possible to measure only the current bypassing the setup. Hence, the damping can be at least partially compensated. For an impedance Z_{el} of the electrode setup and a bypass capacitance C_{by} , the measured impedance Z_{mes} at circular frequency ω is:

$$Z_{mes} = \frac{Z_{el}}{1 + j\omega C_{by} Z_{el}} \quad (4.5),$$

hence, if C_{by} is known, the true impedance Z_{el} can then be found from:

$$Z_{el} = \frac{Z_{mes}}{1 - j\omega C_{by} Z_{mes}} \quad (4.6).$$

Unfortunately, some other structures affecting the measurement are less straightforward to recognize, avoid or compensate. The arrangement of electrode arrays means that around every electrode, several other electrodes are located that can have a considerable impact on current flow and hence impedance. Whereas the behaviour of the electrode/electrolyte interface remains largely unchanged, the flow in the electrolyte can be changed dramatically. This is illustrated by the case of a large field of neighbouring working electrodes connected to the same potential at the same time. Neglecting the gaps between the electrodes, the field then acts as a single, larger electrode. The spreading resistance of the larger electrode is not determined by the electrode area, though. Instead, the resistance scales in a complex manner depending on the overall geometry. In case of a square field of n by n square electrodes, the spreading resistance scales inversely proportional to n , rather than to n^2 as would be the case for dependence on the area. The slower decrease in impedance is explained by a limited ability of the current to spread.

Figure 4.3: Equivalent model for cells covering part of an electrode [68]

For electrodes wider than the distance to the counter-electrode, the current effectively does not spread at all and instead flows approximately in a straight line from electrode to electrode. In practice, it is straightforward to avoid the case of neighbouring electrodes being connected at the same time. Alternatively, the effect can be intentionally used to create a narrower current flow. This method is particularly interesting in that it is compatible with the common design approach to attach unselected electrodes to a defined potential. For large microelectrode arrays, however, the total load from all electrodes may be unfeasibly high.

Even inactive electrodes may contribute significantly to the current distribution in the electrolyte, though, as parasitic couplings exist in the circuits used to select electrodes. Figure 4.4a shows a model of an electrode with a switch connecting or disconnecting it from the sensing line, with a focus on parasitic elements of the switch. The model of the electrode/electrolyte interface is shown as the single element Z_{el} . The switch model has no perfect “on” state, represented by the on-resistance R_{on} in series to the ideal switch. The off-admittance G_{off} indicates that the switch is not perfectly isolating in the “off” state either. Moreover, capacitive coupling to ground is possible before and after the switch, indicated through capacitances C_{sw1} and C_{sw2} . Finally, the sensing line represents a capacitive load as well, leading to capacitance C_{line} . The most critical components are G_{off} , C_{sw1} and C_{sw2} , because they are always present independent of architecture and can only be minimized through the choice of switches. Fortunately, the size of the capacitances and the conductance is negligible for individual switches, and there is no significant effect up to a few 100 electrodes. For larger arrays, however, the cumulative impact can be important nonetheless.

All electrodes in an array can therefore become relevant paths to ground for the signal. This presents a potential problem for EIS measurement, because a T-circuit as illustrated in Figure 4.4b is formed by the spreading resistance R_s in the electrolyte, the interface impedance Z_{el} of the selected electrode and the impedance Z_{par} of the parasitic alternative paths to ground. Such structures are used intentionally to simulate extraordinarily large effective impedances for filter implementations. Because only part of the current driven by the source reaches the end point, whereas the remaining current is leaking to ground, from the perspective of the measurement point the circuit behaves like a larger impedance. For a spreading

Figure 4.4: Impact of inactive electrodes on measurement: a) parasitic elements of a switch; b) T-structure in EIS measurement

resistance R_s , an interface impedance Z_{el} and a parasitic impedance Z_{par} , the measured impedance Z_{mes} can be found from:

$$Z_{mes} = R_s + Z_{el} + \frac{R_s Z_{el}}{Z_{par}} \quad (4.7).$$

Consequently, for small Z_{par} , the observed impedance can be considerably larger than the desired impedance, given by $R_s + Z_{el}$. Moreover, cells can change the impedance of the parasitic path in the same way they can affect the impedance of the path to the selected electrode. Consequently, the parasitic pathway leads to crosstalk between the electrodes.

Parasitic pathways cannot entirely be avoided, but the magnitude and impact of the effect depends on the specific measurement setup. In particular, the situation differs considerably between passive electrode arrays and active CMOS electrode arrays. As no switches are integrated in passive arrays, parasitic coupling on chip is limited to the leads. The external switches have the advantage that they can be measured separately by using dedicated calibration chips. Consequently, it is possible to compensate for the impact, though crosstalk between electrodes remains due to its variable nature. Moreover, it is more feasible to use high quality switches with minimized coupling, since the components do not have to be exchanged. If the array is small enough to select the active electrodes manually, the impact from the contacts is negligible, and only the leads are relevant. The leads can be kept relatively distant from each other and any other wires they might couple to. Hence, the overall impact is low. In contrast, the impact of the leads is negligible in active CMOS electrode arrays, as the electrodes are kept close to the switches controlling the signal flow. However, the parasitic couplings in the switches themselves are considerable. As the switches are realised with transistors in CMOS technology, capacitive couplings across the gate oxide and the p-n junctions are usually dominant, although minor resistive leakage to the substrate is also possible. Because they are part of the same chip, it is not feasible to calibrate measurement to compensate for the impact of the switches. Consequently, even for the same number of electrodes, active CMOS electrode arrays show more parasitic coupling than passive arrays.

If the electrodes are to be selectively activated, the switches connecting them to the instrumentation cannot be avoided. The architecture of the routing network, however, influences the load of the active sensing line, and an example for a poor design is illustrated in Figure 4.5. Independent of the selected electrode, all parasitic couplings are active in this architecture, as the row signal activates a connection to all column lines and hence to all switch capacitances tied to the line. Though omitted in the figure, all capacitors associated with line selection switches are also connected. The majority of capacitors are connected not to the desired signal path, however, but to parasitic pathways that are also activated by the row signal. Furthermore, the connection is facilitated through a large number of switches, leading to a considerably higher conductivity in the parasitic pathways than in the signal path. The change to the current flow in

Figure 4.5: Disadvantageous microelectrode array architecture

the electrolyte cannot easily be modelled analytically, as it depends on too many factors, but neglecting this change, the parasitic path admittance Y_{par} can be estimated as

$$Y_{par} \approx M(N - 1)Y_{open} + (M - 1)Y_{line} \quad (4.8)$$

where M is the number of columns in the array, N is the number of rows, Y_{open} is the parasitic admittance of an unconnected electrode and Y_{line} is the parasitic admittance of an unconnected column line. As the load on the column line is dominated by capacitive couplings from N switches, similar to the capacitive couplings at the electrode side, this can be further approximated for large M and N values as:

$$Y_{par} \approx 2MNY_{open} \quad (4.9).$$

Since all parasitic components are active in the design, it represents a worst-case architecture for electrode arrays. Even in the best case, the open electrodes remain as parasitic pathways and the admittance can be estimated as:

$$Y_{par} \approx MNY_{open} \quad (4.10).$$

Consequently, within reasonable design decisions for active electrode arrays, between best case and worst case there is only a factor of two in parasitic admittance. Nonetheless, the impact

may need to be considered, and it increases with the number of electrodes. Since the parasitic coupling is predominantly capacitive in the model, the impact also increases with frequency.

The importance of parasitic couplings is likely to rise with future developments due to shrinking feature sizes. This is relevant for biosensors, as obtained cell data can only be reliable if the measurements are reliable. For the measurement to be reliable, however, the effects changing its outcome need to be understood and controlled. The mechanisms underlying possible variations in impedance on electrode arrays are complex due to the interaction of many components in a small space. Nonetheless, analytic modelling is possible and indispensable. The complexity of the system may exceed the analytic capability to provide quantitative predictions, and simulations are needed to fill this gap, but even in this case, analytic models remain invaluable for interpretation.

4.3. Electrode and Circuit Behaviour Simulations

Whereas analytic models of a setup are best to understand the behaviour because of their close connection to the underlying physics, they are only feasible for relatively small structures. To capture complex setups, it is therefore necessary to break them down into smaller units. Although in some cases the behaviour of such individual units can be approximated and condensed into a more complex analytic model, it is usually more feasible to determine the interactions between the units in simulations. The transformation into a numerical model loses considerable information, and unphysical results may be obtained, but the complexity of the examined structure is limited only by the available computational resources. Moreover, because the individual components of the model can be localised in a geometric model, visualization of the results is straightforward. Even if analytic modelling of the setup is possible, simulations may be useful tools to derive the required parameters for the modelled case from more general, published material properties.

Obtaining parameters from simulations is especially valuable for complex models with a large number of components that can be estimated simultaneously. Conversely, complex analytical models can also be considered a useful step towards numerical simulations, as the manually created model can retain the most relevant information for understanding of the observed behaviour while determining the complex interactions through numerical techniques. However, finding a suitable model may not be straightforward, and parameters for the components can only be obtained from more complex simulations. Nonetheless, the approach can be valuable if a relationship between properties of the setup and parameters of the analytic model can be found.

The most common approach of finding a model for simulations requires minimal manual intervention and is based on a subdivision of the geometry into small fragments. Within each fragment, an analytical model is constructed from an approximation of the functions describing

the physical processes in the segment, either through discretization or by sums of suitable functions. In either case, the approximation allows the elements and their relationship with neighbouring segments to be represented by a finite number of parameters. These parameters can then be determined using numerical methods. To obtain meaningful results, boundary conditions need to be set for the parameters, and any results are tied to the boundary conditions used. In contrast to analytic models, direct relations between model parameters and properties of the setup can therefore only be estimated through a series of simulations.

A special case for modelling are structures with very high aspect ratio. In the case of biosensors, important examples of this include the electrode/electrolyte interface and cell membranes. Subdividing these structures for modelling either requires an inordinate number of elements or the use of elements with high aspect ratio. However, increasing the number of elements raises the computational effort required for simulation and may exceed available resources, whereas elements with high aspect ratio are associated with high numerical errors. To avoid these drawbacks, thin structures are instead modelled as lower dimensional structures that merely affect the relationship between the elements they separate. The difficulty is hence moved from the partition of the geometry to the formulation of the border condition. Since this derivation can be performed analytically without consideration of the specific application, the computational effort is reduced considerably, whereas the numerical error is determined by the quality of the used border condition.

Another important aspect determining the computational effort of the simulation is the dimensionality of the model. As the geometry needs to be subdivided in every dimension used, the number of elements in the model increases exponentially with the number of dimensions. The impact on computational effort is even higher, since the interaction between elements needs to be considered in calculation. To reduce the number of elements in the simulation, it is beneficial to utilize periodic structures in the geometry and compute the solution only for a single period. Similarly, it is possible to use symmetries to reduce the elements. This is particularly useful in case of symmetry around an axis, as it becomes possible to reduce the geometry from three dimensions to a two-dimensional setup in cylindrical coordinates.

Examples of electrode simulation results from a model utilizing axis symmetry are presented in Figure 4.6. COMSOL Multiphysics was used as simulation environment. Material parameters were taken from literature [51] and adjusted to better match preliminary measurement results. Figure 4.6a illustrates the results for the most basic setup, using a round electrode of $14.1\text{ }\mu\text{m}$ radius, chosen as equal area to a square electrode with side length of $25\text{ }\mu\text{m}$. The size of the electrolyte chamber is limited to $150\text{ }\mu\text{m}$ radius and $100\text{ }\mu\text{m}$ height. These limitations are reflected in the spread of the current, indicated through dashed lines, as the current avoids the lower right edge. Surfaces of equal potential are indicated by solid lines, with the density indicating the magnitude of the gradient. The behaviour of the electrode/electrolyte interface

Figure 4.6: Single electrode simulation results: a) field lines (dashed) and equipotential lines (solid) without cell model; b) field lines (dashed) and equipotential lines (solid) with cell model; c) impact on frequency response

cannot be seen from the graph, as it is represented merely by the line between the electrode, shown in grey, and the electrolyte. Figure 4.6b adds a simple cell model to the simulation, consisting of an ideally isolating hemisphere located $2\ \mu\text{m}$ above the surface of the electrode. This model is meant to represent a cell that has settled on the electrode but does not provide a good seal to the surface. A spherical model floating above the electrode, representing a cell which has not settled yet, would affect the current similarly, although the effect would be less pronounced. The cell model leads to a bottleneck at the corner of the cell, as the current needs to avoid the obstacle. Due to the higher current density, there is a stronger voltage drop across the area, and the equal potential surfaces are more concentrated in the area. The impact of the bottleneck is also visible in the Bode plot in Figure 4.6c. For frequencies above 10 kHz, there is a substantial increase in absolute impedance, though the contrast decreases above 200 kHz. The recorded phase already shows a significant change due to the cell from around 2 kHz. Hence, at least for the simple cell model, a detection using EIS is feasible.

Simulations are also helpful tools for the study of parasitic couplings and their impact on measurement, since they facilitate the study of complex structures. Even for less involved setups, it can be helpful to visualize the influence of design decisions through simulations. Figure 4.7a shows an extended version of the setup from Figure 4.6a featuring a large electrode at ground potential surrounding the working electrode. This is meant to approximate an active

Figure 4.7: Impact of surrounding electrode at ground potential a) field lines (dashed) and equipotential lines (solid); b) impact on frequency response

working electrode in a large array surrounded by electrodes connected to ground. As a result, the current flows almost in a straight line from the counter-electrode to the working electrode and the ring electrode. Merely above a small gap between the electrodes, the equipotential surfaces deviate noticeably from a plane. The surfaces also remain nearly equidistant, indicating an almost constant potential gradient. Because there is no spreading of the current, the observed resistance in the electrolyte is increased. This is reflected in the Bode plot in Figure 4.7b. For low frequencies, the impedance is dominated by the unaffected interface impedance of the working electrode. Around 300 Hz, the phase of the impedance for the setup with ring electrode begins to diverge, pointing to an increasing influence of a resistive component. The absolute impedance is only visibly affected above 4 kHz, but the value settles nearly a decade higher with the ring electrode than without. Hence, at the cost of a significantly increased total current consumption, the current is homogenized and the effective impedance is increased by a factor of 10.

Though these examples provide credible results, it has to be noted that the material and interface parameters the results are based on are rough estimates. As the relationship between the simulation parameters and the observed impedance is complex, matching the values to actual measurement results is difficult. Especially the properties of cells are unreliable, since the impact on impedance may already be hard to detect in the results. Consequently, whereas the general behaviour is representative of the setup, the specific values found for the impedance are inaccurate, and predictions for altered geometries are potentially misleading. Fortunately, reasonable estimates for the behaviour are sufficient for many tasks, and exact model parameters are not required. Visualisation of the behaviour merely requires the general characteristics to be representative. Even optimization of the setup may be possible to a limited degree, though experimental data is required for verification. In any case, the reliability of results obtained from

simulation has to be taken with caution, since unphysical solutions may appear as artefacts of the numerical calculation.

4.4. Summary and Conclusion

The foundations of modelling electrode/electrolyte based EIS setups in both analytical and numerical models are well established. Analytical models are limited in their practical complexity, but they provide opportunities to understand the underlying processes and relationships. They also form the basis for numerical models that can be joined to construct complex models for simulation. The complexity of models for simulation is limited only by the available computational resources and the time required to develop the model. Moreover, techniques exist to limit the model complexity by approximating the setup to reduce the computational effort when necessary. The drawback of numerical processing is that information is lost in the construction of the model, and inaccurate or even unphysical solutions may result.

Consequently, both analytical and numerical models are needed in the investigation of complex systems, and the developed models complement each other well. Simulation results can also be used to obtain estimates for analytical model parameters. This allows more conclusive interpretation of the results, and it also helps to tune the simulation parameters to fit measurement results. However, the limited availability of suitable model parameters is a common problem during model construction. In particular, whereas good models describing the behaviour of electrode/electrolyte interfaces and cells are known, the required parameter values for these models are not always available. Even published material parameters can only serve as estimates due to strong parameter variations depending on the environment and impurities.

Using approximate parameter values, available models are nonetheless of great use for the development of new sensors. Models for individual pairs of electrodes do not consider the environment of the electrodes in depth, however. For microelectrode arrays this presents a significant gap in modelling, as the influence from other electrodes can have a profound impact on the current flow in the electrolyte. For analytical modelling, this influence can be represented through parasitic pathways for the current to ground, leading to a T-structure which functions as a larger impedance in EIS measurement. The impact is even more apparent in simulation results, as the current flow can be visualized, showing a complete change of the current flow. Based on these new models, it is possible to account for the impact of parasitic couplings during design. Though the influence is moderate in current microelectrode arrays, it is bound to grow with the size and bandwidth used, and it may become vital to consider and minimize the parasitic effects during development.

Chapter 5: Design and Evaluation of Electrode Arrays

5.1. Design Options and Limitations of Passive Electrode Arrays

Passive microelectrode arrays are useful tools for many sensing tasks. Since aside from the lead wires, all circuitry is kept off chip, there is no principal limit to the complexity of circuits. At the same time, technological requirements for manufacture of arrays are limited, keeping cost low. Moreover, the simple structure does not contain much space for parasitic coupling. The main obstacle for the application of passive arrays is that the size of the array is restricted by the feasible density of lead wires and external connections. With increasing wire density and length, the parasitic coupling between the wires also intensifies, leading to severe signal attenuation and cross coupling. Hence, if a function can be accomplished with passive microelectrode array, it is generally preferable to select this option over active arrays, but the range of feasible tasks for passive arrays is limited.

Passive microelectrode arrays may be the only option in the development of a sensor if cleanroom capabilities are limited. As the structures used in the array are on the order of at least several μm , the requirements on lithography and etching precision are modest. The fabrication moreover starts with a blank substrate in which no circuits are present, providing controlled starting conditions. Since the substrate merely serves as mechanical support and isolation, minor fluctuations in the material properties also do not affect the characteristics of the electrode array. The start from a blank substrate has the further advantage that there is no need for alignment of the first mask layer. Because a passive electrode array can be constructed with only two mask layers, only one alignment is needed. Moreover, the requirements for this alignment are low, as merely the opening of the passivation above the electrode needs to be placed within its margin. The limitation to one metal layer also contributes to reducing the amount of parasitic coupling. As there are no counterparts above or below the wiring and electrodes, the only nearby conductors are neighbouring structures. Because the structures are relatively large, even the distance to neighbours are considerable, so the coupling is weak. Since there are no circuit elements beside the wiring integrated on passive arrays, this design approach also moves the related parasitic components of these circuit elements off chip. Whereas this does not reduce the parasitic coupling directly, it is possible to use higher quality circuit elements with smaller parasitic components off chip because they can be reused. The array itself, in contrast, has to be discarded in most cases after use due to contamination from the sample.

The drawback to a single metal layer is that the routing capacity is easily exhausted. If the electrodes are arranged in an array of N by M as illustrated in Figure 5.1a, then there are only $2(N + M) - 4$ gaps between the outer electrodes to connect to the inner $(N - 2)(M - 2)$ electrodes. This creates a problem for the case of $N > 4$ and $M > 4$, because the number of

Figure 5.1: Wire congestion in a passive microelectrode array: a) wiring paths towards inner electrodes; b) wire spacing

electrodes grows faster than the number of gaps. For the special case of $M = N$, the number of gaps only grows linearly, whereas the number of electrodes grows quadratically. If there is only one wire per gap, it is not possible to connect all electrodes in an array of 7×7 electrodes. Larger arrays can still be connected if more than one wire is allowed per gap, but the number of wires is limited by the width of the gap, as illustrated in Figure 5.1b. In order to place n wires of width w with a minimum separation d between the wires and from the electrodes, the gap g needs to be at least:

$$g \geq n(w + d) + d \quad (5.1).$$

As the available area for the array is limited, the gap is constrained, though, and the maximum number n_{max} of wires is:

$$n \leq \frac{g - d}{w + d} \quad (5.2).$$

To avoid this limit, it is necessary to increase the number of metal layers. This way, wires can be routed below the electrodes and below each other. However, the required technology for fabrication becomes significantly more complex, as every additional metal layer requires at least two additional masks, one to pattern the metal and one to open contact windows in the separating dielectric layer(s). Moreover, alignment between the layers becomes critical, and the surface needs to be planarised between lithography steps. Hence, the technology necessary to manufacture multi-layer arrays becomes considerably more complex and expensive. Due to the overlap between conductors in different layers, the amount of parasitic coupling is also increased. Consequently, multi-layered passive microelectrode arrays lose most of their advantages compared to active CMOS microelectrode arrays. At the same time, important issues

remain. Independent of the number of layers, for every electrode in the array, a pad is required to connect to the measurement electronics. As the contact pads require a sizeable area, this means that the area requirement for the sensor grows quickly with the number of electrodes. The large number of pads also needs to be connected reliably through the package and socket. Since the sensor needs to be exchanged frequently, the socket is put under considerable wear. This combination of a high number of connections with significant wear presents a major problem for reliability and adds to the cost of the system.

Consequently, passive microelectrode arrays are most suitable if the required number of electrodes is limited and the design can be implemented in a single metal layer. Examples of such designs are given in Figure 5.2. In the example in Figure 5.2a, an array of 81 electrodes is arranged in a square matrix, but only 28 electrodes at the edge are actually connected. This completely avoids problems of wire congestion at the cost of most electrodes serving as dummy. The utility of the dummy electrodes is that a more uniform environment is generated for the connected electrodes. Because no wires are routed between the electrodes, the smallest possible pitch is achieved, which is particularly important for single cell resolution. Moreover, the setup serves to create a situation closer to the regular pattern found on active electrode arrays. Hence, a similar design using 19×19 electrodes was used in this Thesis for comparisons between passive and active CMOS electrode arrays. The design in Figure 5.2b features small working electrodes flanked by large, connected counter-electrodes. By placing the counter-electrodes close and in the same plane as the working electrodes, a compact chamber design is possible. The split of counter-electrode into sections enclosing all working electrodes from two sides ensures a well-controlled potential in the electrolyte, and the aggregate area of the counter-electrode ensures its impedance is negligible. The working electrodes can also be used as sensing electrode in a three-electrode setup to further improve the impedance. A similar setup was

Figure 5.2: Passive microelectrode array structures: a) electrode field without inner connections; b) working electrodes between split counter electrode

consequently used in experiments to determine characteristics of the electrode/electrolyte interface.

As the examples illustrate, there is considerable flexibility in the design for passive microelectrode arrays in spite of the limitations set by fabrication. It remains necessary to restrict the design to a single metal layer in order to take advantage of the positive traits of the technology, though, so the arrays are severely constrained in size. The practical limit in array size turns passive arrays unsuitable for applications requiring a large number of electrodes. If the constrained number of electrodes is appropriate for the task, however, passive design is a highly cost effective way to produce microelectrode arrays on a large scale. Consequently, passive microelectrode arrays remain a sensible default choice for design. Only if the task is not feasible within the constraints of passive technology, the additional costs and issues of active CMOS electrode arrays are justified.

5.2. Advantages and Challenges of Active CMOS Electrode Arrays

By integrating the microelectrode array based on a CMOS process, it is possible to increase the freedom for design considerably. In its most basic form, the addition of transistors allows the architecture to connect selected electrodes to the routing network or leave them disconnected, effectively eliminating the wiring problem. Moreover, it is possible to amplify the signal close to its source and even integrate filters and measurement circuits. Since the electrode array requires a large area at the surface of the chip, which is mostly unused by the CMOS circuits, a considerable area is available for integration. These advantages come at a cost, though, both in terms of financial cost and the complexity of design. Consequently, the step towards active CMOS microelectrode arrays cannot be taken lightly.

Figure 5.3: Routing in an active microelectrode array: a) simple connection matrix; b) hierarchical connection

In spite of the other benefits, the most important advantage of active electrode arrays remains the capability to solve the routing problem, because it makes arrays of thousands of electrodes possible. A straightforward solution for the routing issue is presented in Figure 5.3a. The electrodes are selected in rows, whereas the sensing is done for all columns in parallel. This solution solves the routing very efficiently, as only one switch is needed for each electrode and for each additional row or column, only one further line is required. The design effort is also low, as the regular structure can be repeated as needed. However, the cumulative load on each line leads to considerable problems with parasitic coupling, as explained in chapter 3. The structure shown in Figure 5.3b offers a substantial improvement in this respect. The electrode selection is now combined in rows and columns, and the sensing is organized in regions of the array. The hierarchy of switches resulting from the dual addressing scheme means that despite of using 6 electrodes per region, only three inactive switches contribute to the load on the sensing line. The drawback to this architecture is an increase in design complexity, as more switches are needed and the base cell cannot be tiled as easily as the structure in Figure 5.3a. The ability to integrate complex structures is a strength of CMOS design, though, and the reduction of the routing effort can be pushed further still. The electrode selection can be implemented with far fewer long, shared lines if an address decoder is integrated with each group of electrodes. Similarly, the routing effort and load of the sensing lines can be minimised using a hierarchy of switches. There is, however, a trade-off between the resistance of the switches and the load on the routing network, as the additional depth of the tree adds more switches in series, whereas an increase in width of the tree requires more switches on a single line, adding to the capacitive load.

The trade-off can be circumvented by inserting buffers into the signal path. Though the necessary amplifiers require considerably more area and development effort than the addressing logic and switches used to improve signal routing, the integration with microelectrode arrays is feasible in modern CMOS technologies. Moreover, the early amplification allows to raise the desired signal compared to noise introduced after the amplifier. This is particularly important in applications such as neural interfaces, as in this case noise sources with amplitudes similar to the signal exist very close to the electrode in the form of other neurons. Instead of a signal buffer to counter the load on the transmission path, it is also possible to integrate amplifiers to increase the amplitude of the signal. Based on suitable operational amplifiers, minimal further effort is needed for the construction of active signal filters. With the incorporation of amplifiers and filters, signal integrity can be improved considerably. For designs utilizing high frequency signals and certain implementations of EIS, it may also be useful to include frequency mixer circuits to translate the frequency band of the signal into a range that is easier to process. An important milestone in the process of adding features to the design is the inclusion of analog-to-digital converters, since the digitization eliminates any further corruption of the signal. If the

design effort and necessary chip area is acceptable, it is possible to integrate the entire signal processing chain from signal source to measurement on-chip.

A weakness of the integration is, however, that accuracy is hard to achieve. The variation of circuit elements is significant even within a single chip, and even higher between chips. Hence, although it is in principle possible to include the entire measurement system on-chip, it may not be feasible to do so with sufficient accuracy for the task. The integrated components are also coupled to the substrate and hence with each other. Since the variation of device parameters is a common problem for integrated circuits, techniques have been developed to compensate for their shortcomings. A common underlying principle of these compensation techniques is that parameters of nearby devices on a single chip will diverge similarly from the average. If both devices change exactly proportional, the ratio between them remains constant. This connection can be translated to well controlled behaviour in circuits, as long as only the ratio between two dimensions needs to remain. Through reference circuits, device parameters can also be tuned by adjusting their operating point. However, in biosensors the target of measurement is located off chip, and the electrode/liquid interface presents a major source of variation. Consequently, integrated design techniques are limited in their effectiveness.

The high density of wires and circuit elements in integrated circuits contributes to the strength of parasitic coupling as well, and the effects grow with the size of the electrode array. Besides leading to signal loss, the coupling to other components also leads to cross talk, effectively raising the noise level.

As these drawbacks all need to be considered and mitigated in the design process, the development is complicated significantly. The complicated design effort contributes to development costs directly and also indirectly, because errors in design lead to costly redesign cycles. Every design cycle in itself is considerably more expensive than for passive microelectrode arrays. Besides requiring more manpower and more complex design tools for development, more masks need to be created for each new design. The fabrication for more intricate masks used in the CMOS process is also considerably more expensive than the manufacture of masks for the relatively large structures used in post-processing or purely passive technology. The additional process steps required for CMOS also contribute to an increase in manufacturing costs for each wafer produced. Nonetheless, the overall cost may be in favour of active CMOS microelectrodes if the number of units fabricated is large enough. As the bulk of manufacturing is handled in established foundries, wafer processing can be handled very efficiently. Since post-processing alone uses the same processing steps as a purely passive technology would require, the total cost of a CMOS based technology will remain higher, but the difference is not prohibitive. More importantly, though, the additional costs of the integrated circuits are offset by considerably less expensive external electronics, as specialized components can be kept on-chip. Hence, the overall costs for a solution using active CMOS chips may even

be lower than using passive microelectrode arrays. Moreover, since external electronics are upfront costs, whereas chips can be obtained as needed, the distribution of costs is more appealing from a marketing perspective. However, the cost benefits of CMOS chips rely heavily on economies of scale, and for smaller series of microelectrode arrays, the mask and development costs can become prohibitive.

In spite of the associated high costs, it can be useful to create active CMOS microelectrode arrays with only minimal integrated electronics. These designs serve primarily as stepping stones in the development of more complex integrated circuits to avoid errors. Basic architectures also offer the opportunity to study the impact and demonstrate essential capabilities of the technology with moderate development effort. Such a minimalist design has been developed as a first-generation biosensor and is presented in Figure 5.4, using an electrode addressing scheme similar to that shown in Figure 5.3a. All electrodes in the associated row are connected to their respective column line by a row selector signal. The active column line is then selected by column selector signals. As the selector signals are generated by address decoders, merely 14 address lines are needed to choose an active electrode out of more than 9000 electrodes. Two more off chip connections allow the address decoders to activate or deactivate all selector signals at once. These options cover all important use cases of the microelectrode array, and the electrodes remain accessible with only switches in the signal path. Consequently, the design was chosen for comparisons with passive microelectrode array behaviour in this Thesis. However, although this architecture is useful to study the behaviour and implement all elements necessary to realise an array of several thousand microelectrodes, it is not a good design for an active CMOS microelectrode array meant for field use. As the circuits

Figure 5.4: Active CMOS microelectrode array structure [33]

occupy only a fraction of the available area, the space is not utilized well. The design exhibits considerable pathways for parasitic coupling, though, and the load on the sensing line is not balanced well.

Generally, it is desirable to integrate as many stages of processing on-chip as design effort and available area allow. The use of CMOS technology increases the cost for design and manufacturing considerably, and this increase is only justified if a significant gain is provided. To minimize the errors in the design, though, it is important to restrict the functionality to a manageable complexity. For sensors, it is also necessary to focus the design effort on achieving the required accuracy rather than the range of capabilities beyond essentials like signal routing. However, as even the integration of buffers and amplifiers can make a considerable difference in terms of signal integrity, significant improvements are achievable with moderate effort. In any case, the routing capability of CMOS technology is necessary for microelectrode arrays to be feasible beyond a few hundred electrodes. Overall, active CMOS microelectrode arrays are a costly option, but they offer considerable promise to improve the capabilities of biosensors.

5.3. EIS Measurement Protocol

Two arrays of square electrodes with a common, large counter-electrode were studied in this Thesis. As an example of an active CMOS microelectrode array, a first-generation biosensor with the architecture illustrated in Figure 5.4 was used. The working electrodes were square electrodes with a side length of $22\text{ }\mu\text{m}$ and a pitch of $30\text{ }\mu\text{m}$. A passive microelectrode array of 19×19 square electrodes with a side length of $25\text{ }\mu\text{m}$ and a pitch of $30\text{ }\mu\text{m}$ was selected for comparison. Since the impedance was dominated either by the working electrode or by the spreading resistance, EIS measurement was done exclusively using a two-electrode setup, and no attempt was made to extend the configuration to a three-electrode setup. Phosphate-buffered saline (PBS) buffer with a base pH of 7.4 was used as electrolyte. An HP4284A LCR-Meter was used for all measurements. The observed frequency range was from 1 kHz to 1MHz, and 9 samples were taken per decade of frequencies, for a total of 28 samples. Before measurement, the excitation signal was allowed to settle for 15 cycles.

5.4. Practical Comparison of Passive and Active Sensors

The variety in design possibilities is vast even for passive microelectrode arrays. The options for active CMOS sensors are even more extensive due to the choice of integrated and external components. A meaningful comparison is not always possible, and results can rarely be generalised to different setups. Nonetheless, it is important to evaluate the impact of the choice of technology on measurement. The common unit that forms the base for impedance measurement in an electrode array is the configuration of the electrodes and the electrolyte. Consequently, this unit needs to be similar in the passive and the active CMOS microelectrode

array to preserve a degree of comparability. Even if the geometry and the materials of the central structure are the same, however, the surrounding technology can affect the results, and it is this impact that needs to be investigated. For this purpose, two arrays of square electrodes with a common, large counter-electrode were studied in this Thesis. In these setups, the response is dominated by the working electrode until it falls below the spreading resistance of the electrode.

A comparison of EIS measurement results is shown in Figure 5.5. The results for the passive microelectrode array largely follow the expected trend, with the absolute impedance falling about 20 dB/decade until near 1 MHz when the impedance starts to settle around 15 k Ω . There is also just a negligible difference between the case where the signal is applied on the counter-electrode (top driven) and the current is measured at the working electrode and the case where the connections are reversed (pixel driven). In contrast, the active CMOS measurements show a significant deviation from the basic electrode/electrolyte characteristic. The absolute impedance is more than a decade higher than for the passive array, and the pixel driven case differs considerably from the top driven case. In particular, despite a substantially lower absolute impedance, the pixel driven measurements show less consistent results for frequencies below 20 kHz. Moreover, for both the top driven and the pixel driven case an increase in impedance is observed around 300 kHz before a steep decline above 500 kHz, which has no equivalent in the passive array results.

These results cannot be explained by a simplistic model interpreting the electrode setup as a two-terminal device, as the signal amplitude is too small to generate significant non-linear

Figure 5.5: EIS Measurement comparison between active CMOS and passive microelectrode array

behaviour. Moreover, the rise in impedance for the active CMOS microelectrode array is too large to be caused by geometric factors alone. The increase can be explained, however, using the T-circuit model introduced in Chapter 3, if a considerable parasitic path is assumed. A capacitive component to the resistive path also explains the rise in impedance, as the current leakage and hence the observed impedance grows with frequency. The steep decline at high frequencies, in contrast, suggests a capacitive path bypassing the T-structure. The asymmetry in observed impedance is not explained directly by the T-circuit or the bypass capacitance, but it can be explained if the parasitic path introduces noise to the measurement. If the noise is inserted with a smaller impedance to the counter-electrode contact than to the working electrode contact, the impact of the noise is stronger in the pixel driven case. This is the case for low frequencies, since the total impedance is dominated by electrode/electrolyte interface of the working electrode.

While the results for the passive microelectrode array were relatively stable, those for the active CMOS array varied strongly from chip to chip and from electrode to electrode. In particular, the bulge in the characteristic at high frequencies changed considerably in its clarity, onset and cut-off frequency. In part, the deviation in behaviour could be traced back to design flaws in the CMOS chip. The sensor used very long connection lines without any kind of compensation, and the signal switches were too narrow, both of which is adding to the impact of parasitic coupling. Nevertheless, the observed variation from chip to chip supports the parasitic character of the observed effect, as the strength of parasitic couplings depends on fluctuations in the process that are badly controlled. However, the difference in observed impedance for low frequencies was so large that an error in handling had to be considered. Especially, it would have been possible that by accident an electrolyte with lower conductivity was used. Even so, although this could also explain a considerable change of impedance, it cannot explain the asymmetry of measurement results for the active CMOS array.

To investigate further, the experimental setup was expanded with a known resistor in series to the impedance formed by the microelectrode array. Assuming a T-circuit consisting of a series of resistors R_A and R_B and a leakage admittance Y_L , an added resistor R_S in series to R_A changes the observed impedance Z_{mes} to:

$$Z_{mes} = (R_A + R_S) + R_B + Y_L(R_A + R_S)R_B \approx Y_L R_A R_B + Y_L R_B R_S \quad (5.3)$$

Hence, the series resistor R_S can change the observed impedance by more than its own value, whereas in the absence of a T-circuit, the change is equal to R_S . As expected, measurements on an active CMOS microelectrode array with a series resistor showed a noticeable increase in impedance even for values as low as 10 Ω . For passive microelectrode arrays, the impedance increased as well, though more pronounced for higher frequencies, which was consistent with a T-structure formed by the array, the series resistor and the capacitance of the probe used to contact the chip. The drastic increase in impedance found on the active CMOS

array, however, indicated a considerable current leakage that was not explained by the structures on-chip. Moreover, the chip design suggested primarily capacitive coupling which would have been stronger at higher frequencies, explaining the intermediate rise in impedance but not the increase for low frequencies. The cause of the strong leakage was finally identified as an insufficient isolation in packaging.

Even after correction of the packaging error, a difference in measurement results between active CMOS and passive microelectrode arrays remained, though considerably smaller. Whereas the lower impact of parasitic couplings appears more representative for practical designs, the stronger leakage serves to emphasise the underlying effect. Even in its weaker form, the current leakage cannot be neglected, since it increases with frequency due to capacitive coupling. For lower frequencies, however, the impedance is dominated by the interface characteristics of the microelectrodes, which do not carry the relevant data for biosensors. Moreover, the characteristics of all microelectrodes in an array are similar due to identical geometry and materials, independent of activation. Hence, the parasitic coupling into inactive electrodes increases together with the desired coupling into the selected electrode. As the effect grows with the area of the electrode array and the capacitive coupling increases with frequency, the impact from parasitic couplings is likely to increase with future development and needs to be considered. As the diversity of architectures for microelectrode arrays is large, though, it is impossible to find truly representative samples, and results cannot directly be applied to other designs.

5.5. System Considerations and Architecture

The microelectrode array is a central part of the measurement system, and its integration with other system components needs to be considered. This is particularly true for active CMOS microelectrode arrays, since it is necessary to decide which sections are integrated with the array. The required components depend on the chosen measurement approach, particularly if an active or a passive method is utilized. Essential sections for EIS measurement are presented in

Figure 5.6: Components of an EIS measurement system

Figure 5.6. As an active measurement approach, the technique first requires an interrogation signal to be generated. The signal is then applied to the driving electrode of the array, usually the counter-electrode. The next component in the signal flow is the core function of the microelectrode array, the working electrodes and the routing network with selection of the active electrodes. Assuming a voltage excitation is needed, a transimpedance amplifier (TIA) is then required to convert the resulting current into a voltage that is more straightforward to process and decouple the input from the further processing. This may be followed by a signal filter, but a more common approach is to digitize the signal with an analog-to-digital converter (ADC) and apply filters in the digital domain. A necessary module for EIS measurement is a demodulator or fast Fourier transform (FFT) to extract the frequency components from the response. Finally, the digital output needs to be stored for retrieval by the control unit. The control unit is also responsible for configuration of the system, and it serves as interface between the measurement system and external components like a PC or user interface.

For the signal source, it is most important that the spectral distribution of the interrogation signal is known. In most cases, this is achieved by accurately generating a constructed signal with the desired properties, though in principle, it is sufficient to track deviations by accurate measurements. This option is important for on-chip integration, as it may be useful to reuse an ADC block that is anyway required for signal evaluation. In the most straightforward case, the interrogation signal consists of a single sinusoid. Whereas this signal is easy to understand, it is not trivial to generate accurately. A reliable way to produce the signal with predictable accuracy is to construct the signal shape in the digital domain and use a digital-to-analog converter (DAC) to translate it into a usable form. This also makes it easy to synchronize the signal generation with the signal demodulation, but it is limited in its frequency range by the necessary oversampling of the DAC.

As an added advantage to constructing the signal in the digital domain is that a superposition of multiple sinusoids is straightforward to implement. By applying multiple frequencies at once, it is possible to speed up the measurement considerably. A special case of using multiple frequencies is the use of periodic signals other than sinusoids. Since these signals can be Fourier transformed to a sum of sinusoids with known amplitude and phase, they can be used as interrogation signal. The signals may, however, be considerably easier to generate. The easiest signal to generate reliably is a rectangular signal, as the processing can be done completely in the digital domain, and only a one bit DAC is required to scale the signal to the desired range. With minimal additional effort, however, it is possible to use more complex digital sequences with a more desirable spectral distribution. Whereas an ideal, flat distribution cannot be achieved by a digital sequence, optimized pseudo-random bit sequences (PRBS) can be found that approximate this behaviour well [69], improving measurement speed at high bandwidths considerably.

Whereas the digital interrogation signals are easier to integrate with the required accuracy than analog signals, though, the higher bandwidth makes signal evaluation more difficult. The bottleneck for evaluation in this case is the ADC, which needs to be able to follow the signal and introduces digitization noise. Higher resolution reduces the impact from noise, but requires more complex designs which are harder to design with the required sampling rates and occupy a much larger surface area on the chip. In case of a single sinusoid interrogation signal, the relevant signal has an extremely small bandwidth, which means that only a small fraction of noise cannot be filtered out of the digitized signal. The more frequency components are used simultaneously, the harder it becomes to design satisfactory filters and demodulators to suppress the noise. For high bandwidth signals, such as for PRBS interrogation signals, filters cannot achieve significant noise suppression without averaging over several signal periods, effectively cancelling the speed benefit of the high bandwidth signal.

To obtain meaningful phase data on the impedance, it is essential that the interrogation signal is synchronised with the demodulation or FFT. If the signal is constructed digitally, the synchronisation is straightforward, and full integration on-chip is helpful to achieve predictable timing. Demodulation is feasible only if the interrogation signal comprises few frequency components, as each frequency requires separate processing. FFT is more flexible, but it requires more effort than single demodulators. Moreover, FFT can only be implemented reliably in the digital domain, whereas demodulators can be realized with analog circuits. Consequently, the demodulation can be applied before digitization, lowering the requirements on the ADC considerably. Analog demodulation is hard to integrate with high precision over a wide frequency range, though, and the overall effort may be in favour of placing the ADC before the demodulator. Nonetheless, it is necessary to demodulate the signal first if the ADC cannot provide the required conversion rate. Moreover, a reduced sample rate is easier to transfer the data off chip directly.

Independent of the data rate, it is preferable to integrate the ADC on-chip, since it avoids signal degradation during transfer. In principle, digital data transmission is feasible up to far higher data rates than the high-resolution ADC is able to provide. Digital filters, demodulators or FFT needed for EIS processing do not take up impracticably large chip area, though, and on-chip processing allows considerably lower transmission rates. Since the demodulation or FFT needs to be synchronised with the signal source, it is also useful to integrate both together in spite of the increased on-chip area requirements. All components of the measurement pipeline in between need to be integrated in this case as well, as a transfer off chip and back is not practical. A complete integration of the measurement pipeline does require considerable chip area, but a sizeable area is available on-chip under the border of the electrode array that needs to be kept free for the walls of the microfluidic chamber. Moreover, the inclusion of all modules is beneficial from a financial viewpoint as well, since it avoids the need for exotic external

components. Only the control unit benefits from an implementation split between the sensor and the supporting electronics, since the necessary data transmission gains considerable flexibility and speed if it makes use of a commercial microcontroller. Because every integrated module means significant development effort, however, incorporating the entire measurement pipeline may require several generations of sensor designs. Whereas it is generally desirable to include all modules on-chip, it is feasible only if all individual components have been tested.

5.6. Summary and Conclusion

Both passive and active CMOS microelectrode arrays have their own benefits, and the better choice depends on the task at hand. Whereas passive arrays feature a cheaper, less complicated technology and offer superior performance in terms of electromagnetic compatibility, active CMOS arrays are more scalable and are able to incorporate more functionality. Conversely, passive arrays are limited in their applications primarily by practical constraints on the number of electrodes that can be integrated, whereas the main limitations for active microelectrode arrays are related to design effort and cost efficiency. Hence, passive microelectrode arrays are preferable for tasks requiring only few electrodes, and active CMOS microelectrode arrays are preferable for tasks requiring a large number of electrodes. The border between tasks more suitable for passive arrays and tasks more suitable for active arrays is fuzzy, though, and determined more by economic than technological factors, a decision can only be made on a case by case basis.

From a purely technological point of view, active CMOS electrode arrays improve on most aspects of passive electrode arrays. The most important enhancement is that the routing problem underlying the electrode limitation of passive arrays is eliminated. By integrating more functional units on-chip, the design of external electronics is also simplified considerably. Moreover, the integration of signal buffers and ADCs close to the electrodes improves signal integrity, as the area exposed to noise is reduced. At the same time, however, although the high density of circuit elements reduces the distance for parasitic coupling, the transistors in a typical CMOS technology are optimized for speed rather than reduction of parasitic components. Hence, active CMOS microelectrode arrays may suffer from increased current leakage and noise injection due to parasitic coupling. To prevent this, design efforts need to consider and mitigate the effects of parasitic elements as far as possible. If the active CMOS microelectrode array is designed accordingly, however, it is possible to integrate a high-quality measurement system with few external components.

Chapter 6: Selective Biosensors for Biological Cell Capture and Analysis

6.1. *Cell Capture on Blank Electrodes*

The analysis of cells is an important task for biosensors. For the analysis of cells to be reliable, the conditions of the measurement need to be well controlled. This is not only important for the quality of measurement itself, but also in order to ensure that cells are kept alive. Even if death of the cells is acceptable for the given task, it is necessary to ensure the death occurs in a controlled manner. Consequently, great care needs to be taken during manipulation of cells. In this case the quality of measurement results is essential, but it is also more forgiving in that variations in measurement conditions can be partially compensated for. However, additional sensor input is required to estimate the conditions needed for correction.

In some cases, the additional information can be obtained from baseline measurements. An important example of the utility of baseline measurements is the estimation of electrode interface properties before the actual measurement. This is important because the interface characteristics may vary considerably. Other important factors like the temperature of the setup can be acquired with sufficient precision by spatially separate sensors which do not interfere with the biosensor itself. Neither of these options is viable, though, for the location of the cells under investigation. Consequently, it is important to limit the variation of the cell location during measurement. In case of flow-through setups, this means that the cells need to be kept within a defined channel within the microfluidic channel. For stationary setups, unless the cells can be held steadily in place by other means, they need to settle on the surface.

The straightforward approach to ensure stable positioning on the surface is to allow for the cells to settle by themselves after the original deposition. This method is viable in laboratory, but it takes considerable time and predicting its value is hard because the attachment and spreading of a cell on the surface is a slow process that can take hours from onset to completion. It is not trivial to keep cells alive during this process. Besides a favourable temperature, a supply of nutrients and removal of waste needs to be ensured. Among these nutrients are dissolved gaseous components such as oxygen and carbon dioxide, which are easy to exchange but take considerable effort to keep in well defined supply due to their volatile nature. At the same time, loss of moisture due to evaporation needs to be minimized to prevent a harmful concentration of salts in the serum. A practical solution is the use of an external incubation chamber for storage of the sensor during cell immobilization. However, the system can then not be self-contained, and trained personnel is needed for maintenance of the incubation environment.

In order to speed up the settling process and gain better control, it is necessary to apply force to the cell. A possible method which has been successfully demonstrated utilized microfluidics,

letting the cell be dragged along with the flow until it gets caught in an orifice too small for it to pass [49]. A major advantage of microfluidic cell capture is that it is intuitive to understand and comparatively easy to track during development. However, the capture presents a considerable strain on the cell, and the suction generating the strain is difficult to control resulting in a high danger of damaging the cell. Moreover, the manufacture of channels required for microfluidic flow is not always fully compatible with CMOS technologies. Polymer channels can be formed on top of the chip in post-processing, but the channels are subject to routing problems similar to the wires leading to the electrodes. As every opening in the channels needed to capture a cell leads to a considerable loss of pressure, multiple capture sites cannot easily be operated with a combined access channel. Combination with high density microelectrode arrays is hence only viable if the channels are etched through the wafer, opening space for a large pressure chamber, but also requiring a back etch and greatly limiting the space available for circuitry.

An alternative solution to establish a hold on the cell is to utilize Dielectrophoresis (DEP). By applying a suitable alternating electric field, a force is created drawing the cell towards the field. If the field originates from an electrode, this effect can be used to pull the cell onto the surface. Since only electrical signals are required, DEP can be implemented together with the measurement circuits. As a result, DEP has successfully been used together with EIS for the detection of beads [42]. However, the voltage levels to create appreciable force are significantly higher than those used for sensing, and dedicated high voltage CMOS technologies may be required. Moreover, the force created by DEP depends on dielectric properties of each cell. In the case of a complex sample made of several cell types, the very strong electric fields can lyse certain cells. Hence, to make use of the effect, the circuits and electric field magnitudes used need to be tuned to the cells under investigation.

Thus, although methods are known to control and accelerate the settling of cells, the effort for their integration with measurement circuits is considerable. Without external forces, cells may not settle on the electrodes as desired. Even if a cell is positioned on an electrode, the electrode is not necessarily covered well. As illustrated in Figure 6.1, the displacement has a substantial impact on current flow and hence the observed impedance. The cell in Figure 6.1a has settled well centred on the electrode and only a small current is able to escape at the edges, whereas the cell in Figure 6.1b is positioned on the edge of the electrode, allowing a much larger current to escape.

Figure 6.1: Effect of cell location on current flow: a) centred cell; b) cell on edge of electrode

giving a high impedance. In contrast, the cell in Figure 6.1b cannot block the current effectively, since it has settled on the edge of the electrode, and the observed impedance is considerably lower in spite of similar cell characteristics. Even when cells have not settled on the surface yet, the location of the cells relative to the electrodes has a significant influence on the observed impedance. This has been investigated in more detail for the case of Coulter-counter type structures in [70].

In stationary systems, the close proximity and the irregular shape of the cells make it difficult to find good estimates for the influence of cell position on impedance measurement, though the impact is considerable. However, even without the use of external forces, it is possible to affect the settlement pattern of cells. As living organisms, cells are capable of movement. Even if the range of migration is limited, it can be influenced by patterns on the surface. This has been demonstrated using regular structures patterned on the chip surface with periods between 0.1 and 10 μm and corresponding depths between 0.01 and 1 μm , with the cells aligning to the orientation of the structures [71]. The general factors that influence the cell reaction to a surface patterned topography are reviewed in [72]. Though the alignment of cells is straightforward to show, the mechanism behind the effect is less clear. For the purpose of this Thesis, it is sufficient to consider that cell alignment is related to movement patterns, and that the movement along a surface is hampered by trenches.

In the manufacture of microelectrode arrays, trench-like structures are formed automatically either between the electrodes, or the electrodes themselves are located in cavities. Consequently, it may be beneficial to consider the effect of the surface on cell settlement. Even if the topography is merely an artefact of manufacturing and not meant to be utilized, the understanding of cell settling on the chip is improved. More importantly, if cell settling is affected negatively by the topology, an understanding of the underlying mechanism may aid in optimizing the geometry of the design. Figure 6.2 illustrates the basic ways in which the cell can be affected by substrate structures with protruding electrodes. Such electrodes are easier to manufacture with good seal in post-processing than electrodes in trenches due to alignment issues and are hence more important for active CMOS microelectrode arrays. To emphasize the impact of the structure, the trenches shown in the figure are deeper in relation to the cell size than is to be expected in a real setup: the typical diameter of cells is on the order of 10 μm , but the height of the trench wall surrounding the electrode is unlikely to exceed 2 μm , since the deposition of material is time consuming.

The ideal case for the alignment of cells is shown in Figure 6.2a. The cell first touches part of the exposed substrate or electrode. While settling on the surface, the cell stretches sideways, but the movement stops at the edge of the trench. Ultimately, the cell is centred on the surface and covers the area without extending significantly beyond the plateau. However, the movement may not be stopped by the edge as in Figure 6.2b. Instead of filling the area of the plateau, the

Figure 6.2: Possible settlement behaviour of cell on patterned substrate: a) cell settles on top of a protruding surface; b) cell spreads across the edge into a cavity; c) cell spreads across a trench; d) cell settles in a cavity

cell then spreads into the trench. The cell may also descend between two electrodes. If the trench is narrower than the diameter of the cell as in Figure 6.2c, the cell may attach on both sides of the chasm and bridge the gap. If the opening is wide enough, however, the cell can descend to the bottom as in Figure 6.2d. The cell then spreads and attaches to the floor and walls while filling the gap, though small open spaces are likely to remain. With less deep trenches, the cell may also be able to reach the top of the wall and extend further on the plateau, though the edge still forms an obstacle. If the electrodes are located at the bottom of cavities, the behaviour from Figure 6.2c or 6.2d becomes the ideal case, as the cell seals the opening and blocks the current. An incomplete seal similar to Figure 6.2b may in this case also occur if the cell reaches the bottom of the cavity and extends across some of the edges. The worst-case for electrodes at the bottom occurs if the cell settles across a wall between electrodes, as neither of the electrodes is sealed, but the measurement is still affected.

The actual settling behaviour and movement of the cell is not determined by the structure alone, but rather depends on the type of cell and the material of the surface. Hence, extensive studies on the targeted cell type are needed to optimize the surface topology for a particular cell

line. Moreover, even cells of the same type react differently to the same circumstances. This limited predictability is a major drawback to cell capture on blank electrodes, since the uncertainty about cell location significantly affects the quality of measurements. However, the passive capture may be the only feasible option due to the considerable additional efforts for both design and manufacturing needed to ensure that cells are placed right on top of the electrodes. The guidance using a passive patterned surface structure is then an invaluable tool to improve the cells' settling behaviour. None of the described methods avoid the need to keep a hospitable environment for the cells. The capture even relies on the health of the cells, either to move and attach itself to the electrode, or to withstand the forces applied to draw the cell into place. Hence, the environmental conditions remain the most important factor for cell capture, in spite of advanced methods to control the capture process.

6.2. *Surface Modification for Cell Capture Control*

The attachment of cells to the surface relies on the ability of adhesion proteins on their membrane to bind to the material of the surface. Consequently, the materials used for the surface have a substantial impact on the process of cell capture. The adhesion proteins expressed by different types of cells vary considerably, so the interaction with the substrate also changes significantly. Cell types such as red blood cells which circulate freely in the blood stream tend to exhibit fewer adhesion proteins. This reduces the danger of getting attached to other cells in the stream or the blood vessel itself, but it also affects the ability to capture the cells. Hence, the cell types under investigation may affect the materials usable for the design of a suitable sensor. Moreover, the interaction between the cell and the materials of the substrate is complicated by the interaction between the cell and the topology of the surface.

Material variations at the surface are an inevitable aspect of electrode arrays. Conductive materials are needed for the electrodes, whereas an isolating material is needed to separate the individual electrodes. Due to the manufacturing process, the materials are also linked with topographical features of the surface. This allows for combining the effects of topography and material differences to reinforce the impact on cell adhesion and settlement. Unfortunately, whereas the topography can be controlled by design and manufacturing steps, the choice of bulk materials for the electrode array is limited by manufacturing and electrical requirements. Metals are the material of choice for electrodes due to their high conductivity and interface characteristics, and cell attachment is not a main concern in standard CMOS processing. Many cells nonetheless attach well to biocompatible metal surfaces such as Au or Pt, but not significantly better than to the surrounding isolators such SiO₂. However, it is not necessary to change the bulk material to affect the cell behaviour, since the adhesion proteins attach only to the surface. Hence, it is sufficient to modify the surface chemistry. As Chen et al. reported, it is

Figure 6.3: Comparison of electrode and isolating area

even possible to reduce the adhesive area of a substrate to the point that instead of spreading, the cell initiates apoptosis through a patterned surface coating [73].

While it is generally undesirable to enforce cell death in this way, the ability to prevent cell attachment to an area is of great importance to microelectrode arrays. This is illustrated by a comparison between the area used for electrodes and the area needed between electrodes as shown in Figure 6.3. For an electrode pitch of 30 μm and square electrodes with a side length of 20 μm, more than half of the area is used for the isolation. Since cells are descending approximately uniformly over the entire area, this means that on average, more than half the cells will begin to settle on the area between the electrodes. Only the parts of the cell that cover the electrodes can be detected and analysed well, so the proportion covering other areas poses a problem for detection. The straightforward solution is to increase the electrode size compared to the electrode pitch. This approach is limited by technological requirements, however, and the side length of the electrode would need to be 95% of the pitch for a square electrode to reduce the separating area below 10%. Since it is not feasible to prevent the cells to descend onto the isolation, it is important to promote their migration on the surface onto the electrodes. As blank electrodes offer sufficient adhesion for many cells, it may be more important to reduce the adhesion on the isolator instead. Patterns of reduced adhesion for cell guidance have been successfully created on quartz as early as 1992 [74]. Similar techniques can also be used on microfabricated electrode arrays. Moreover, since the oxide used for isolation is chemically similar to quartz, whereas the electrodes are substantially different, it may be possible to achieve the desired surface modification without the need for a pattern mask. The caveat to the presented method is that the surface is turned hydrophobic to achieve the non-adhesive behaviour. This poses a problem for filling the microfluidic chamber above the electrode array, hence this approach was not pursued in this work.

In contrast to the need to prevent adhesion on non-electrode surfaces in microelectrode arrays, the promotion of adhesion in certain areas of the chip is only necessary to perform dedicated tasks. An example is the study of cells expressing insufficient adhesion proteins. Because these cells do not attach to the blank metal electrodes, additional effort is required to

ensure their capture of the cells. Circulating tumour cells are likely to fall under this category, since they have detached from their tumour source and float freely in the blood stream. A more important reason to actively promote adhesion through surface modifications is, however, that it allows to improve selectivity. By engineering the surface properties to bind well to specific adhesion proteins, it is possible to promote adhesion for cell types that strongly express those proteins. Other cell types show a different expression of adhesion proteins, and hence their attachment is not affected in the same way. In the ideal case, adhesion is promoted for the desired cell type, whereas it is impeded for competing cells.

Since adhesion proteins are involved in the formation of multi-cellular organisms, for many proteins there are appropriate counter proteins available to which they bind in the body. Another natural option to find a suitable counterpart to proteins is to use an immune system reaction to produce antibodies to the targeted cells. The main advantage of these approaches is that the proteins are highly specific to the cells they bind to. Moreover, appropriate macromolecules already exist in the body or are straightforward to manufacture. However, the proteins can only be manufactured through biotechnological means. Hence, the quality and properties of the product varies considerably between manufacturers and individual charges. The complex compounds are also highly sensitive to processing conditions and prone to degradation over time.

Fortunately, smaller molecules exist which also bind selectively to surface proteins of cells. These less complex compounds are more stable and can be synthesised by purely technical means, avoiding the major drawbacks of the proteins. In the course of synthesis, it is also possible to equip the smaller molecules with functional groups, aiding considerably with their attachment to the surface. Important groups of small molecules that can be used for cell capture include peptides and aptamers. Peptides are defined through the peptide bond connecting short chains of amino acids. An example of peptides used to promote adhesion of pancreatic cancer cells is given in [75]. Aptamers typically consist of short chains of nucleic acids, but they are defined through their ability to bind selectively to a target. Hence, the definitions of peptides and aptamers are not mutually exclusive, and molecules belonging to both groups are called peptide aptamers.

Figure 6.4: Adhesion promotion through dedicated surface layers: a) complementing and incompatible surface functionalization; b) too densely packed surface

The selective binding mechanism for short chain molecules and proteins is largely the same and illustrated in Figure 6.4a. The proteins in the membrane of the targeted cell type A have a characteristic shape and characteristic chains of amino acids. The molecules deposited on the electrode to promote adhesion match the shape or characteristic chains to form a tight bond. Cell type B predominantly exhibits different proteins in their membrane, so the bond formed to the surface is weaker. The sparse coverage of the electrode with an adhesion promoting molecule as in Figure 6.4a does not prevent cell type B from attaching to the surface, however, since the adhesion proteins can still attach to the uncovered surface. The cell may also weakly express the characteristic membrane protein used to promote binding to cell type A, contributing further to the bond. A dense coverage of the electrode with an adhesion promoting molecule as in Figure 6.4b on the other hand is better at preventing undesired bonding, but it also reduces the flexibility and accessibility of the binding group. Consequently, even though the molecules of surface and cell membrane match, adhesion may be diminished.

Since accessibility of the key groups of the adhesion promoters is vital for binding, it is not helpful to add more thickness to the promoting layer. Moreover, since the utilized compounds are bad conductors, an excessive coverage of the electrode is detrimental for the charge transfer to the electrolyte. In contrast, a thin layer does not block the current and may even aid with charge transfer without compromising the adhesion quality. Consequently, the ideal case is a well aligned single layer of the adhesion promoting compounds on the electrode. Such a monolayer is not feasible to manufacture by direct deposition such as printing, because the amount of the compound cannot be controlled with sufficient precision. Moreover, a self-aligning process is needed to cover the entire electrode without affecting the area around the electrode.

The desired molecular monolayer can be deposited by using the self-alignment properties of adhesion promoters. By furnishing the components with suitable functional groups at a defined site, they gain the ability to form a chemical bond with the targeted electrodes, whereas no such bond is formed with the surrounding area. A particularly useful example is the thiol group, which reacts with metallic surfaces to form metal thiolate complexes. Since the reaction only occurs at the location of the functional group, the orientation of the compound is constrained. Once a sufficient density of the compound is reached on the surface, the molecules also bind laterally with each other, reinforcing the orientation. As the reaction with the surface is needed to capture the molecules out of solution, there is no further deposition once the surface is covered. Hence, a self-assembled monolayer (SAM) is formed on the electrodes. If synthesized molecules are used for adhesion promotion, they can already be prepared with the appropriate functional group during synthesis. Proteins, on the other hand, need to be functionalised in an additional manufacturing step by attaching a short chain molecule with the desired functional group. Moreover, since proteins are very large molecules, even a single layer

can have a considerable thickness. The alignment within the layer is also affected. Consequently, the synthesized molecules are generally preferable for the formation of adhesion promoting SAMs.

Even with the support of chemical bonds, the flat electrode is not an optimal surface for a cell to adhere to. The planar structure also undesirably minimizes the available area for charge transfer at the electrode/electrolyte interface. Therefore, it is desirable to change the surface structure to augment the opportunities for the cell to hold onto and improve the current exchange. The most straightforward way to increase the surface area of the electrode is to increase its roughness by etching. This is impractical for microelectrode arrays, though, because the resulting porosity of the surface is prone to expose lower levels of the material stack. More promising is the immobilization of carbon nanotubes [76] or nanoparticles. These nanostructures considerably increase the surface area and hence improve the interface characteristics. Metal nanoparticles are moreover capable of surface modifications with SAMs as in [59], combining the advantages of nanostructures with the advantages of adhesion promoting molecules. However, the cost of these advantages are additional processing steps and the corresponding process variations.

For sensors using electrode arrays, a lack of uniformity in interface is a major problem, because the variations over space turn into systematic measurement errors. SAMs using synthesized molecules are potentially useful in this respect, as the monolayer can provide a well defined surface. In particular, the metal coverage provides protection against corrosion and stabilizes the surface. However, the formation of the SAM itself is highly sensitive to the state of the underlying surface before the treatment, since the anchoring functional group needs to be able to react with the metal. Any residue on the surface will interfere with this process. The environment during the SAM formation can also introduce variations of the layer quality. Nanoscale reactions that are used to produce nanostructures are inherently hard to control with sufficient precision to allow for uniform properties. Although this can partially be compensated for by filtering and separating the products into different bins, the underlying variation affects the homogeneity of the formed layers. Hence, neither nanotubes nor nanoparticles are likely to provide a uniform interface behaviour over a microelectrode array.

Due to the difficulties involved in the application of surface modifications, considerable research is still needed for surface modifications to become available as regular tools in biosensor design. Their potential for increasing specificity and improving electrode characteristics is substantial, however. In particular, the combination of surface design with the topological characteristics inherent to microelectrode arrays offers promise for improved cell capture and analysis. The most feasible approach for this integration is currently the use of SAMs from synthesized molecules, though nanostructures are likely to bring about further improvements once variations from production are reduced. A significant benefit of limiting the

design to utilize only SAMs is that the combination with topological features is straightforward, as the formation occurs only on the targeted surfaces. Hence, the surface properties can be aligned with the topological effects in the guidance of cells to the electrodes. However, process requirements remain an issue even for basic surface modification, and suitable SAMs for specific tasks need to be found.

6.3. *EIS Measurement with Surface Modification*

Surface modifications can have a profound impact on EIS measurements. The most intuitive change is introduced by modifications aiming to increase the surface area of the electrode, as the larger area translates to a reduced electrode impedance. However, since the characteristics of the electrode/electrolyte interface depend on material properties, even thin and sparse SAMs may change the behaviour considerably. The details of this influence are beyond the scope of this work, but it is important to note that surface layers can affect the impedance of the electrode in several ways. The molecules used for surface modification are isolators and consequently present an obstacle for current from the electrode. Similarly, the monolayer is inserted between the charges of the double layer at the interface, increasing the distance and reducing the capacitance. Conversely, molecules used for the formation of SAMs may exhibit a considerable dipole moment, improving the coupling and increasing the capacitance. If the molecules in the SAM are polar, they may also be able to bind ions in the electrolyte, increasing the effective concentration near the surface. The boosted concentration allows for higher surface reaction rates, reducing the transfer resistance. Hence, the surface layer may improve or deteriorate the interface characteristics significantly, or the underlying mechanism may change without observable changes in the measured response.

The same impact that has to be considered when utilizing SAMs with EIS based sensors also makes EIS measurements a useful tool for the study of SAM formation. Other measurements may be needed for verification, though, because the changes to the observed impedance cannot be clearly attributed to a particular mechanism of action. The nature of the corroborating measurements depends on the goals of studying the SAM. An important tool in the study of surface modifications is a measurement of the contact angle of a chosen liquid. Though this test reveals only a single feature of the surface, it is significant because it provides explicit information about the surface. However, reliable measurement requires a sufficiently large, uniform surface area to do the measurement on, which may not be available on microelectrode arrays due to the small dimensions of the electrodes. Detailed studies of small areas can be done using scanning microscopes such as tunnel microscopes, but are not feasible for regular tests due to the necessary equipment and effort. Consequently, EIS measurements are an attractive substitute during process development, whereas more specialized measurements are reserved for more in-depth studies.

For EIS based sensors, the tracking of the impact of surface treatment on the electrode impedance is the most significant test. The details of the surface layer are of minor importance in this case, as beside the impedance, only the functionality of the surface is relevant to the task. EIS measurement is also suitable to track and possibly even control the deposition of SAMs, because it provides most of the relevant information and can be done quickly and repeatedly with little effort. If the SAM is deposited from a conductive solution, EIS measurements can even be taken during the process, avoiding the need to remove samples from the bath for testing. The caveat of this approach is that measurement may affect the creation of the monolayer, though due to the low energy levels used for EIS, the probability of a major impact is low. Consequently, a conservative approach to SAM formation requires that EIS measurements are only done separately to avoid any possible interference, and this approach was chosen in this work. Nonetheless, it appears likely that on-line measurements during processing will be the superior approach in series production.

A possible problem when using EIS to test SAM preparation is that at least two terminals are needed for measurement. If both terminals are located on chip, the SAM will be formed on both electrodes, and the change in impedance will affect both electrodes as well. The measurement results show only the superposition of both changes. The superposition can be compensated for if both electrodes have the same properties, but if there is a difference in geometry or the SAM is not formed uniformly over the electrodes, an error is introduced. The straightforward solution is to keep the second terminal off chip and only introduce it immediately before the measurement. This introduces errors due to uncertain geometry, however, and the properties of the second terminal may vary as well. Moreover, the microfluidic chamber and counter electrode for applied measurements need to be mounted before SAM preparation because the manufacturing conditions would otherwise destroy the SAM. Consequently, there may be no access for an additional test electrode. A better solution is a three-electrode setup compensating for the transfer function of one of the electrodes. The measurement then shows only the change of impedance for the electrode that is not part of the control loop. However, a suitable location needs to be reserved for the sensing electrode to ensure reliable control. Hence, a retreat to the two-terminal measurement may be preferable despite the tangled results if only qualitative statements on the SAM progression are required.

As the microfluidic chamber needs to be completed before SAM preparation, the monolayer also affects all terminals involved cell detection or analysis. However, the impact in this case is lower, since the impedance of the electrodes themselves serves merely as baseline for measurement. The ideal result of the surface modification in this respect is hence to lower the baseline. Unfortunately, a major decrease of electrode impedance can only be achieved by a significant increase in electrode area, which can only be provided by the more complex application of nanostructures. Since SAMs need to be selected primarily for their ability to bind

to the target cell, a minor decrease in electrode characteristics may be inevitable. The expected benefit in using SAMs remains only in the ability to improve cell capture and bond between the cell and the electrode. An exception to this is that the bonding event between the cell and the adhesion promoter may induce a charge shift in the molecule. This shift is too small to detect reliably using EIS, however, and more sensitive measurement techniques are required. Nonetheless, the impact of the SAM on the electrode performance needs to be considered for EIS based sensors, and a major increase in impedance due to the SAM is not acceptable.

An increase in baseline impedance is rarely desirable for EIS based sensors, because the values tend to be high already before modification. Fortunately, it is possible to find surface modifications such as SAMs for many tasks that affect the impedance only moderately. In particular, for a given modification, the process parameters can be optimized to yield a compromise between impact on the electrode performance and the desired bonding effect. Moreover, with the future development of reliable application of nanostructures, the electrode characteristics can be improved significantly. In the meantime, EIS and SAM formation present a promising combination of tools, with EIS being both the final goal and a valuable tool for investigation.

6.4. Experimental Methods

The objective in the context of this Thesis was a preliminary feasibility study rather than an in-depth investigation, because the surface modification represented only a branch of the sensor development. Moreover, the SAM formation strongly depends on the surface prior to the treatment, which is subject to change. Hence, experiments were limited in scope and merely meant to demonstrate the feasibility of the approach. As the final goal of the surface modification was an improvement in selectivity and cell capture, the molecule forming the SAM was chosen accordingly. The selected adhesion promoter is an aptamer that binds to the Epithelial Cell Adhesion Molecule (EpCAM) [77]. Since EpCAM is a protein strongly expressed in the membrane of most solid cancer cells, it is a highly suitable target to promote adhesion of most CTCs to a functionalized surface. In this Thesis, an MCF-7 cell line was used as a representation for EpCAM expressing cancer cells. The protein is, however, also expressed in healthy epithelial cells, whereas some CTC lines do not express the protein. Accordingly, EpCAM is not a suitable marker for all cancer types, but it has nonetheless already become an important adhesion promoter for a subset of cancer types [23]. False positive reactions to a surface modification targeting EpCAM are also unlikely in an enriched blood sample, because the expression of adhesion proteins in regular blood cells is low.

Before the preparation of a SAM, it is imperative to ensure the treated surface is free of contaminations that could affect the layer formation. For this work, the final cleaning scheme began with an acetone wash for 5 min. The acetone was replaced by an IPA wash lasting for

another 5 min. The IPA was then removed by a triple rinse with deionized (DI) water before drying the chip using a stream of nitrogen. The dried chips were cleaned further using an O₂ plasma treatment. These cleaning steps had to be done before mounting a polymer based chamber on the passive glass chips, as the acetone and the plasma treatment damaged the polymer and the reaction released new contaminations. After the chamber assembly, only an ozone cleaning step for 20 min remained. For the active CMOS chips, the formation of the chamber was completed before the cleaning, but the microfluidic network leading to the chamber was omitted since it was damaged by the cleaning procedure.

The aptamer solution was deposited immediately after ozone cleaning. To facilitate this, the solution was prepared in two stages. The aptamers were obtained from Sigma-Aldrich in crystalized form, and a 100 μ M solution in ultrapure water was prepared. Following manufacturer directions, heat treatment was used to activate the aptamer and break up undesired thiol-thiol bonds. The solution was then aliquoted into charges of 5 μ l and stored at -20°C. The diluted aptamer solutions that were used for surface treatment were then only prepared from the aliquots when the chips were undergoing ozone cleaning. This way, both the chips and the aptamer solution were ready just in time for the surface treatment. The aptamer concentration suitable for surface treatment was estimated from similar work in the literature [78, 79, 80, 81, 82] and previous work in our project [83], before settling on a concentration of 1 μ M. The required time for incubation was originally estimated from the same sources, but in order to study the formation of the SAM, a range of incubation times from 30 minutes up to 100 hours was tested. Since the electrodes are hence subject to soaking in water for a considerable time, a separate set of chips were treated with DI-water for control.

EIS measurements were done immediately after SAM formation to avoid deterioration. Two chip designs were used for the measurements, one passive design and one active CMOS design. The majority of measurements were done using passive chips, as the response was considered more representative of the actual interface characteristics. The working electrodes on these chips were square electrodes with a side length of 25 μ m, whereas the counter electrode was a large, coplanar electrode that was divided to spread between the electrodes. For the comparative measurements on active CMOS microelectrodes, first generation biosensor chips were used. The instrument used for all EIS measurements was a Metrohm Autolab PGSTAT 302.

To verify the SAM effect on cell attachment, experiments with two cell lines were done. Since the tested aptamer is binding to EpCAM, MCF-7 cells which express this protein were chosen as the positive cell sample. For control experiments, NCI lung cancer cells that do not express EpCAM were selected. This control was imperfect, because the cell lines differ in several aspects besides the EpCAM expression. Moreover, the MCF-7 cell line has been modified to express a fluorescent protein. However, since the binding mechanism of the SAM relies on its structural match with EpCAM, the results remain meaningful. In any case, a perfect

control for cells is nearly impossible to achieve, as the complex interactions in cells' biochemistry means that any change can have considerable unforeseen consequences. The concentration of cells in the test samples was controlled by manual cell counting and according dilution or enrichment. The adjustment was merely used to adapt the expected density of cells on the chips, though, and no exact count was recorded, since only patterns of cell settling were evaluated. Chips were seeded with the cell culture immediately after preparation of the SAM and stored in an incubation chamber at 37°C. After a first storage of up to 24 h in the incubator, the serum was replaced and unattached cells were removed using fresh nutrient solution. The chip with the sample was then stored for at least another 24 h in the incubator, before rinsing the chips with PBS and staining the cells for investigation under the UV microscope.

6.5. SAM Formation on Microelectrode Arrays

The impact of a surface modification on the electrode/electrolyte interface is as complex as the underlying surface chemistry. Though the basic mechanisms have been identified, the specific outcome can vary significantly due to impurities in the used materials. This bestows considerable weight on experimental results in the assessment of surface modifications. Consequently, investigations were needed to support the use of surface modifications with microelectrode arrays. Outcomes of experimental studies are uncertain, however, and substantial experience is needed for the preparation of surface modifications. Nanostructures, in particular, offer great promise in improving electrode characteristics, but were excluded from this work due to uncertainty about suitable recipes and expected results. Within the scope of this Thesis, investigation was limited to the formation of SAMs on the electrodes in order to focus the available resources on a promising approach for use with microelectrode arrays.

The study of the surface treatment was concentrated on the impact on impedance measurement, as the procedure was intended for an EIS based sensor. Consequently, the main questions to be answered were how the electrode characteristics would be affected by the treatment and how basic process parameters would influence the outcome. In particular, it was necessary to ensure that the electrode impedance is not increased beyond the scale caused by inevitable degradation. Only if the EIS measurement remains a feasible route for cell detection after SAM preparation, the binding functionality can be used to improve measurement.

First experiments were done using passive electrodes manufactured on a glass substrate. This avoided the complication of the measurement response from an active chip and gave a clear indication of the impact of the SAM on impedance. The passive chips also had a less complex structure, making it easier to trace measurement results to the active electrode for optical comparison. The passive structure had the further advantage that all electrodes were directly accessible, making the realization of both two and three electrode setups possible through external connections. Consequently, the passive electrode arrays were the preferred choice for

Figure 6.5: EIS measurement results for passive microelectrode array during SAM formation

EIS experiments. In initial contact angle measurements, however, the surface structures were too small to fit the water droplets with sufficient margin, and the results could not be considered meaningful. The number of electrodes on each array was also lower on the passive arrays, and the electrodes had to be selected by hand. Since each EIS measurement cycle required several minutes, the feasible number of scans was limited as well, though, and the time required for manual selection was negligible.

The observations were hence mostly qualitative rather than quantitative in nature. Even for qualitative statements, further experiments are needed to verify the results. However, the obtained measurement results for passive microelectrode arrays exhibited low variance between electrodes of the same chip, indicating that a good estimate of the true impact was possible. Results for measurements using Au electrodes and 1x phosphate-buffered saline (PBS) solution as electrolyte are shown in Figure 6.5. For a short treatment, such as an aptamer incubation for 1 h, only a marginal increase of less than 1% for most of the spectrum in absolute impedance was observed compared to the control, though changes were visible in the phase domain. Only after long incubations times of more than 4 h, the impedance change became distinguishable clearly from chip to chip variations. Even for a 100 h incubation, the absolute impedance grew by a factor of three. The results obtained from the two-electrode setup were matched by measurements using a three-electrode configuration. This was unsurprising, as the large counter electrode had an impedance that was negligible compared to the impedance of the working electrode. Beside the desired impact on electrode characteristics, the results also illustrate weaknesses of the measurement setup. Around 50 Hz, the impact of noise coupling from power lines is visible, showing a weakness in shielding. Moreover, the apparent decline in impedance

beyond 30 kHz is largely explained by coupling between the wires from the EIS instrument to the device under test. In spite of the weaknesses of the setup, the results were sufficient to show that the SAM formation did not excessively degrade electrode performance. Whereas the increase for very long incubation times may be problematic due to the small signal to noise ratio for cell detection, it does not present an unsurmountable obstacle, particularly if the impedance is stabilized at the higher level.

The impact of the SAM formation was also investigated on active CMOS microelectrode arrays using first generation biosensor chips. These experiments were primarily meant to bridge the gap between previous results on the biosensors and the newer results on SAM formation obtained from the passive electrode arrays. It was particularly useful to see the reaction to the surface treatment on the first-generation biosensor because Chen had previously shown that cell detection was possible using the chip [33]. If the electrodes were not degraded by the SAM, this capability would be preserved, since the detection relied merely on the unaffected change of impedance within the electrolyte due to the cell. The experiments were also needed, however, due to the different surface condition. As the processing of the passive array and the active biosensor were different, both the material of the electrode and the surface structure are different, possibly affecting SAM formation. A big advantage of the active CMOS microelectrode arrays was that they could be configured such that no electrode was selected. This allowed for a baseline measurement capturing the characteristics of the admittance bypassing the sensor and thus limited compensation in the results. Hence, measurements provided valid results up to 1 MHz. Unfortunately, only a small number of unused biosensors was available for tests, and reuse of the chips was problematic due to the danger of changed surface characteristics. Even without reuse, the microelectrode arrays displayed considerable chip to chip variation, and no reliable chip to chip comparison was possible. Consequently, the experimental procedure had to be adapted. Instead of leaving the aptamer solution in the microfluidic chamber for the entire incubation time, the treatment was interrupted for measurements. To facilitate this, the chips were triple rinsed with DI-water before applying the electrolyte solution. After measurement, another triple rinse was applied before inserting fresh aptamer solution. This way, the progress of SAM formation could be followed, though the change in procedure made results vulnerable to errors in handling and there was no direct comparability with results from the passive microelectrode array.

The results obtained with the active microelectrode array are consistent with the passive case insofar as the surface treatment had only a minor impact on the observed impedance. The trend was, however, less clear, as illustrated in Figure 6.6. After a temporary decrease, the absolute impedance increased again. The phase remained largely unaffected up to 1 kHz, but thereafter also displayed a change that was reversed with higher incubation times. This behaviour may have several possible explanations. A detrimental surface condition may have

Figure 6.6: EIS measurement results for active microelectrode array during SAM formation

persisted after the cleaning process which was slowly dissolved during the SAM formation, before the impact of the dense layer followed the same pattern as in the case of the passive microelectrode array. This appears unlikely due to the intense cleaning protocol, but inorganic contaminations may have remained. It is also possible that the aptamer obstructed narrow parasitic pathways such as porous oxides before beginning to affect the electrode characteristics. However, the parasitic effect appeared less pronounced in the chips used for aptamer experiments and was apparent only in phase behaviour. Since the surface properties before treatment were different from the parasitic microelectrode array, though, the effect of SAM formation may also be different, with sparse layers promoting charge exchange whereas dense layers block the current. Lastly, the observations may have been an artefact of the changed experimental procedure, as the intermediate cleaning steps may have damaged the SAM. More detailed studies are needed to clarify. In any case, no critical electrode deterioration was noticeable. Hence, the capability for cell detection remained largely unaffected.

Overall, the obtained experimental results showed considerable variation from chip to chip and between measurement series. The suspected sources of this inconsistency are contaminations remaining after cleaning or electrodes which had already degraded during storage of the chips. Consequently, the cleaning process needs to be investigated further. In particular, resting stages may have to be included in the cleaning protocol, since contaminants may be released only over time. Moreover, future designs of the microfluidic chamber need to take the cleaning into account to avoid material incompatibility. Once an updated cleaning protocol has been established, the investigation of SAM formation will need to be verified and expanded. Unfortunately, the necessary resources for the required experiments have not been

available. The common observation, however, was that in all completed experiments, no detrimental impact on electrode impedance was found that could critically impair the cell measurement capability. Hence, the aptamer SAM is suitable for use with EIS on microelectrode arrays.

6.6. *Cell Culture and CTC Capture with SAMs*

The most important part in the study of a SAM is to show that it can accomplish the functionality it is supposed to provide. The objective in case of the biosensor under investigation is to promote the adhesion of CTCs to aid with capture and thus detection. Accordingly, it is necessary to show that CTCs are successfully attracted to settle on the electrodes, whereas other cells remain unaffected by the surface modification. To study the impact of the SAM on cell adhesion, cell cultures need to be deposited on the treated substrate and allowed to settle. The experiments need to be done for both cells that should be affected and cells that should not be affected by the surface modification. In case of the cells targeted by the SAM, an alignment of the attachment pattern to the treated surface structures is expected. Cells that do not exhibit the targeted adhesion protein, on the other hand, should not show any alignment, and ideally even adhesion is limited by the surface treatment.

Before cell culture experiments, the chips were prepared following the protocol described in section 6.4. Immediately after preparation, the chips were seeded with the cell culture and stored in an incubation chamber at 37°C. The cells were given considerable time to settle after seeding, as fast attachment to the substrate was not considered an objective at this point. The cells must be kept alive during the entire procedure until observations have been recorded. The chamber the cells are held in therefore needs to allow for proper handling of the cell culture, e.g. exchanging the serum without drying out the cells. Even in open lid designs the forces affecting the cells are hard to control, risking severe damage to attached cells. This effectively rules out setups relying on capillary forces to fill the chamber, since the fluids cannot be removed from the chamber without considerable force. However, as no EIS measurements were needed for cell culture evaluation, it was also not necessary to use fully functional packages for the chips. Instead, designs without fixed lid were used, giving easy access to the chamber. It was even possible to use dummy chips that had been manufactured by applying the post-processing procedure on unprocessed wafers. Besides considerably reducing the chip costs, this also had the advantage of reducing the topological influences on cell orientation, since the thin layers deposited in post-processing introduced only minor unevenness to the surface. The disadvantage of the dummy chips was that the surface properties are not identical to the fully processed CMOS biosensors. Hence, the results were not necessarily representative, though the impact of the differences in surface characteristics is lower on cell attachment than on EIS results.

An example of early cell culture results using MCF-7 cells on treated dummy chips is given in Figure 6.7. The cell line used for these experiments was modified to express a fluorescent protein, so staining was not necessary for UV microscopy. The aptamer had been incubated on the chip for 2 h, and the cell culture was kept in the incubator for 24 h in the first storage period and for 36 h in the second storage period. The cell culture was seeded at high concentration to nearly fill the entire area of the chip, though a considerable part of the cells was removed during serum replacement. Figure 6.7a shows a composite from images taken of a sector of the electrode array in bright field and blue light excitation conditions. The cells spread widely on the substrate, many covering the surface of multiple electrodes. A substantial portion of cells died or were in the process of dying after the long storage, which can be seen in the weak fluorescent response. At first glance, no order is visible, as the cells tend to touch their neighbours. At closer inspection, however, many of the cells' borders align with the edges of the electrodes. Figure 6.7b shows a composite image taken of a sector at the edge the electrode array, with seven larger squares which cover the bond pads in a fully processed chip. Whereas the distribution is similar on the electrode area, the cells have no structure to align to on the oxide area, and the settlement pattern without guiding influence can be seen. In Figure 6.7c, an image of the fluorescent response on a different sector of the electrode array is shown. An area with particularly good alignment is highlighted and shown at higher magnification. Without the bright field image, the electrodes are illuminated only by the fluorescent glow from the cells,

Figure 6.7: Settling pattern on treated substrate at high cell concentration: a) composite image of cells spreading on the array; b) composite image of cells at the edge of the array; c) blue excitation image of cells aligning with array pattern

but the alignment can still be clearly seen as the cells spread over several electrodes and along the nearly straight borders of the cells.

Though the initial experiments succeeded in demonstrating the alignment of cells to the grid presented by the electrodes, further investigations were needed to show that the aptamer SAM was responsible for the alignment. In particular, control experiments with EpCAM negative NCI lung cancer cells were used to test if they were showing different adhesion patterns than the EpCAM positive MCF-7 cells. Figure 6.8 shows results for a 2 h surface treatment with aptamer solution. The cultures were seeded with a considerably lower cell concentration, kept in the incubator for 12 h in the first storage period and for 24 h in the second storage period. In Figure 6.8a, a sector of the microelectrode array is shown with well aligned MCF-7 cells outnumbering badly aligned cells. The figure also illustrates the construction of the composite image. The bright field image clearly shows the pattern of the electrodes, but cells are appearing merely as shadows. The fluorescent response to blue light excitation on the other hand highlights the location and health of cells. Only the composite image clearly shows the quality of alignment between cells and electrodes, though due to the strong orientation, patterns are also recognizable in the fluorescent response. A higher magnification composite from the same sector is shown in Figure 6.8b. Out of the seven cells in the image, six are aligned near perfectly to the electrodes, though the cells do not appear to have spread to fill the available area. Figure 6.8c shows a composite image of a sector of the electrode array with NCI cells. The cells do not show any clear alignment to the electrodes, but rather exhibit a strong tendency to remain in clusters of cells. The NCI cell line used is not modified to express a fluorescent protein, so fluorescent imaging relies solely on the staining of the cells. As this stain affects primarily the nucleus of the cells, the apparent size of the cells is significantly smaller in the composite than in case of

Figure 6.8: Settling pattern on treated substrate at low cell concentration: a) composite image realisation; b) cells aligning with electrodes; c) control cells settling on the array

the MCF-7 cells. The same stain has also been used with the MCF-7 cells in the experiments, but the information obtained from observation of the nucleus has little meaning for cell alignment.

Whereas the observed alignment of the cells to the substrate structures was not consistent, it was clearly enhanced by the surface treatment. In light of the varying behaviour of living cells and the preliminary nature of the experiments, the observed improvement exceeded expectations. Unfortunately, too few chips were available to produce comparable, quantitative data, and it is unclear how far the results can be generalized to CMOS biosensors due to the differences in surface characteristics and topology. The topological characteristics in particular offer substantial opportunity for further improvement of cell alignment, though. It also appears promising to combine the gains from the aptamer SAM on the electrodes with a repulsive layer on the oxide to prevent cells from attaching between the electrodes. Nevertheless, even the aptamer alone enhances the alignment and selectivity of cell adhesion, offering considerable improvement for reliable cell detection and analysis.

6.7. *Summary and Conclusion*

Cell capture is a crucial aspect of cell detection on stationary biosensors, as the location of the cell has a considerable impact on the measurement response. The capturing process is affected by surface characteristics such as the topology and material properties. Hence, it is valuable to consider these features in the development. Besides providing an insight into the settling behaviour, these studies offer the prospect to optimize cell capture with dedicated surface modifications. Especially SAMs of molecules attaching selectively to proteins specific to targeted cells hold promise to improve both cell capture and ability to discern between different types of cells. Suitable molecules can be synthesized to bind to many membrane proteins, though finding the appropriate sequence may not be straightforward for some tasks. If the group responsible for the binding is known, however, it is possible to create doubly selective molecules, binding selectively both to a specific type of cell and to only certain materials on the surface of the chip. This allows for mask-free processing with ideal alignment to the surface structures. SAMs are also an excellent match for EIS-based sensors, since the thin layers do not affect the interface characteristics severely. Nonetheless, due to the remaining effect the SAM has on the observed impedance, EIS may still be useful in the study and especially monitoring of layer formation.

In this chapter, a novel application of an aptamer binding to the EpCAM molecule on microelectrode arrays has been investigated. As the surface properties have been studied only from an application point of view and the process parameters have not been optimized, the results have to be considered preliminary and further research is needed. Even within the limited scope of this work, however, key results have been achieved. The prepared SAMs provided

significant improvements to selective cell capture and alignment with only minor electrode degradation. In future developments, these enhancements may be further compounded by combination with an intentional use of topology and repellent surfaces to guide the cells towards the electrodes for investigation. Although no increase in signal strength for cell detection with EIS has been shown yet, the enhanced cell alignment and bonding to the electrode provide important advantages for the detection process. In any case, the improvement in selectivity alone justifies the use of the aptamer SAM, and the relatively low effort in processing does not present a major obstacle.

Chapter 7: Second Generation CMOS Array Characterization

7.1. *A Biosensor with Integrated Amplifier Chain*

Parasitic couplings, the identification of their sources and minimizing their effects present a significant challenge for large microelectrode arrays, as discussed in chapters 4 and 5. To reduce the impact of the consequential current losses on measurement, the parasitic couplings need to be taken into account during design and measurements, and compensated for as much as possible. The signal source needs to be able to drive the load to be measured and all active parasitic paths, which may present a significantly larger load than the measured impedance by itself. As one of the major sources of parasitic couplings are long, common lines, these need to be avoided where possible. One way to achieve this is to insert buffers. This is particularly important close to the electrodes, since the relevant input to the electrodes has a relatively large impedance. The amplifiers near the electrodes are still driving the common lines, so their output impedance must be minimized. The input of the amplifier needs to provide a good virtual ground when active, and yet also must be well decoupled from the electrode when inactive. Inactive electrodes should not be grounded due to the large current they may draw. This presents an important caveat, as grounding is a common technique to ensure a defined state of the electrodes.

In the field of bioelectronics, circuit design practices require particular clarity. Due to the interdisciplinary nature of the field, not all researchers have a meaningful background in electronics. It is, however, important to communicate the problems the design techniques are meant to solve. Moreover, standard design practices need to be questioned. The interface to the biological segment has properties that are unusual in pure circuit design, so the standard IC design conventions may not apply. In the case of microelectrode arrays, the electrolyte chamber creates a connection between all exposed electrodes at all times. A common counter electrode strengthens the link, since it runs in parallel to the long, thin extension of the electrolyte that would otherwise present a large impedance. This interconnect is the reason that grounding the electrodes is not a feasible choice in microelectrode arrays. Other biosensor designs may lead to different problems with design techniques. Nonetheless, best practices from integrated circuit design are an important factor in minimizing parasitic couplings.

The impact of good design techniques was demonstrated in the second-generation biosensor. This biosensor had been designed by collaborators before the problems with parasitic couplings became apparent, and the main goal was to integrate components of the measurement system. The biosensor was also designed to allow flexible, parallel measurement of electrodes. For these reasons, the design comprised an elaborate electrode selection scheme and amplifier chains to buffer the signal. As base process for manufacturing of the biosensor, a Global Foundries 0.18 μm CMOS technology was used. In post-processing, a 1 μm gold layer was added to cover the

electrodes for biocompatibility. The technology offered a compromise between high integration and analog behaviour of the transistors. In particular, the process provided a number of different MOS transistor models with different channel lengths and an operating voltage of 3.3V.

The structure of the biosensor is shown in Figure 7.1a. The design features an array of 104×104 microelectrodes to achieve a total of 10000 electrodes, even when rows or columns at the edges are covered by walls of a microfluidic chamber due to alignment errors. The pitch of the electrodes was $30 \mu\text{m}$ as a compromise between process limitations and the required single-cell resolution. The array is divided into two halves to increase the number of parallel readout channels. Because input amplifiers need to be shared between multiple electrodes due to area requirements, the electrodes are grouped in pixels of 4×4 electrodes each, giving a structure of 26×13 pixels per half array. The pixels are grouped together in columns along the shorter axis. Hence, 26 readout channels are available per half array, for a total of 52 channels. However, only 4 of the 26 channels per half are connected off chip at any given time because the number of available output pins is limited. Each pixel comprises a transimpedance amplifier (TIA) with adjustable gain. Each column provides an amplifier in unity gain configuration to buffer the TIA output.

Figure 7.1b shows the structure of the adjustable TIA. The purpose of the adjustment is to adapt the amplifier to different impedance ranges. This does not require fine tuning of the amplification, but rather a rough setting. This is accomplished by inserting a series of ten $100 \text{ k}\Omega$ resistors into the feedback loop that is partially bypassed with transmission gates in parallel to adjust the feedback impedance. Instead of, or together with the resistors, a capacitance of 0.86 pF can be also used in the feedback path. The operational amplifier at the core of the TIA consists of a PMOS (p-channel metal-oxide-semiconductor) input stage with a folded cascode,

Figure 7.1: 2nd generation biosensor a) overview; b) TIA design; c) zoomed image of pixel

followed by a class-A output stage. The design is very compact and fits easily in the $120\text{ }\mu\text{m} \times 120\text{ }\mu\text{m}$ space of a pixel. However, a cascode Miller compensation is needed to stabilize the amplifier. In the technology used for the biosensor, the needed capacitor utilizes the top metal layer that is also used for the electrodes. In order for the capacitor to fit in the layout, it was split into 16 parts that are arranged in cross shapes between the electrodes. These structures are clearly visible in the zoomed image of the pixel in Figure 7.1c.

Selected electrodes are connected to the input of the TIA with transmission gates, whereas unselected electrodes are left floating. The output of the TIA is coupled to a common column line if one of the electrodes is selected. The selection of multiple electrodes in one column at the same time is not intended, though there is no protection against it built into the chip. If any of the electrodes in the column is selected, the column buffer is activated. The buffer uses a similar structure to the operational amplifier in the TIA. Besides being used in a unity gain configuration, the only major change was an expansion of the input stage to complementary differential pairs, providing a rail to rail input range.

An additional pixel without exposed electrodes is integrated for test purposes. Instead of an electrode, the selected TIA input is connected to a known, external impedance. The test impedance reflected an equivalent circuit model for the electrode/electrolyte interface and consisted of two capacitors and two resistors. The test circuit also includes a column amplifier, though only the test pixel is connected to the input.

7.2. Characterization and Design Lessons

The majority of characterization were done on the test amplifiers because they are readily available through pins of the chip. In contrast, the microelectrodes of the array are only accessible using a probe station. Accordingly, they were only used for functional test of the chips. The functional test starts with a test of the addressing logic. Once it is assured that individual electrodes can be selected, activation of the electrode TIA is tested. Only once this is certain, a test signal is inserted through a known test cell and the probe into the electrode. Since the electrodes need to be contacted manually with the probe, only few electrodes were tested in this way.

Following the specification, the test signal was a single 50 mVp-p sinusoid input voltage. The frequency of the signal was varied in 10 steps from 1 kHz to 1 MHz. The output of the amplifier chain is also a voltage and was measured on an oscilloscope during tests. The results for dry tests, shown in Figure 7.2 reflect the ratio of the feedback impedance to the input impedance. Although the feedback impedance is configurable, the biosensor was only characterized using the full chain of feedback resistors with the parallel capacitor activated. In simulations, the sensor achieved output levels of 31 mV to 387 mV under these conditions. The measurement results matched the simulation well, with deviations well below the expected

Figure 7.2: Simulation and measurement results for second generation biosensor

tolerance of 10%. Hence, the simulation results for the frequency response of the amplifiers was fairly accurate. The gain bandwidth product (GBW) of the TIA was found to be 24 MHz and the GBW of the column amplifier was 33 MHz, well above the required bandwidth of 1 MHz.

All tests were dry tests without an electrolyte on top of the electrodes. Consequently, the results are not fully applicable to the study of parasitic couplings, as the current distribution in the electrolyte is a major factor. This is expected, since the design pursued other goals. Nonetheless, it is possible to judge the architecture in light of the lessons learned from the first-generation biosensor. Moreover, the tests exposed weaknesses of the design that need to be considered for future development. Although the reduction of parasitic couplings was not an explicit goal of the design, the second-generation biosensor exhibited improvements. The grouping of microelectrodes to pixels sharing a common TIA was done because the circuit required too much area to fit under a single electrode. The compact arrangement in groups of 4×4 rather than in a single line of 16 electrodes was chosen in order to ease routing. At the same time, however, this architecture reduces the wire length before the amplifier and consequently the parasitic couplings from the wires. This is particularly important for the unselected electrodes that are left floating. Long lines are not entirely avoided in the design, since it was not a design goal. The shared line from the TIAs to the column amplifier is unproblematic as long as collisions are avoided in electrode selection, since the output of the TIA has a low impedance and the coupling is negligible. The long resistor chain presents a more serious possible issue, since with parasitic couplings the arrangement forms a T-structure with the potential to severely distort the response of the circuit. This did not lead to observable

problems in the given architecture, but it needs to be considered in the development of new designs.

A weakness of the biosensor was the driving strength. The specification of the design was for impedance values from 10 k Ω . However, these specifications were derived from measurement results on first generation biosensors. Because these results may significantly underestimate the load due to the impact of parasitic couplings, the specification may not reflect the true requirements. While the feedback impedance of the TIA could be adjusted to fit the output voltage within the operating range of the amplifier, the driving current was limited by the class-A output stage. Increasing the current is possible, but the impact on the frequency response of the amplifier poses a considerable design problem. Moreover, the power consumption creates problems for heat management and, if many amplifiers are used in parallel, also for the power supply.

Independent on the amplifier structure, the parallel measurement of many electrodes is a potential cause of problems for EIS-based biosensors. To achieve the parallel sensing, a common counter-electrode is driven by the interrogation signal, and all selected electrodes measure the ensuing current. However, this effectively synchronizes the current flow over the entire chip. Consequentially, the design presents a highly fluctuating load, creating difficulties in ensuring a constant power supply, both on- and off-chip. As this was never tested on the second-generation biosensor, it is unsure how critical this issue would become in practice. Nonetheless, the issue of balancing the load needs to be taken into account for new designs. In any case, it is useful to note that the synchronisation is created in this form due to the common counter electrode and the measurement approach. The large counter-electrode is needed to ensure its influence on the measurement is negligible. The approach driving from the counter electrode and measuring at the working electrode is the most common implementation of EIS and used in commercial equipment. More importantly, it is also a way to allow parallel measurement at multiple electrodes. For these reasons, the second-generation biosensor was designed using this approach, and the structure is not suitable for alternative measurement topologies. In particular, the TIA is only useable as a current sink. In the context of the design goals of the biosensor, this drawback is irrelevant. However, the observation is valuable for future development.

The biosensor was also designed for measurement only using a single frequency signal. This was a consequence of the available measurement equipment, which only offered this option. However, measuring the impedance at multiple frequencies in parallel is an important way to speed up sensing. Since the required bandwidth and linearity of the design are unaffected, using multiple frequencies has no impact on the amplifiers themselves. The configuration of the feedback structure of the TIA is useful only if the impedance for all observed frequencies are similar, though. Similarly, the biosensor is not designed for use with digital stimuli. The cutoff

frequencies of the amplifiers are sufficiently high, but the steep edges of digital stimuli may exceed the slew rate.

Another issue not addressed by the specification or the design was manufacturing tolerances of the circuits. Since the feedback circuit of the TIA uses on-chip resistors and capacitors, the amplification is subject to the variation of these components. The switches to adjust the feedback lack the necessary resolution to compensate for the deviation. Moreover, it is not feasible to connect the electrodes to known impedances for calibration. Limited correction is possible by using the test amplifiers for reference measurements and scaling the final measurement results, but a mismatch between the test structure and the actual pixels remains unaccounted for. Calibration of the sensor is not necessary, though. Since the presence of cells is detected by a change in impedance, the actual value of the impedance is not relevant for detection. An important consequence of this is that a biosensor design can show very large variations between samples without affecting its ability to detect cells. However, baseline measurements without cells are necessary to attain the relative change of impedance.

Although the internal design of the TIA was suitable for its purpose in the biosensor, there are important consequences for the performance of the chip as well. In particular, the compensation capacitor affects the behaviour. Besides improving the frequency response, it also draws current at the output. This does not lead to immediate problems, because the output stage was designed taking the load into account, but it adds to the problem if the total load was underestimated. Because of the topology of the TIA, the capacitor is not directly connected to the electrode. This is helpful because the additional load does not affect the output voltage of the amplifier and also because the amplifier is shared by several electrodes. If the capacitors use the same metal layer as the electrodes though, as is the case in this biosensor, this leads to problems with the placement of the capacitors. Splitting the capacitor and placing the parts between the electrodes is an elegant solution, but it affects the surface topography of the chip. This may create problems for post-processing and influence the settling of cells on the surface in an undesirable manner. From the perspective of parasitic coupling, the placement at the surface also means that the capacitors are coupling to the electrolyte. Noise and signals picked up from the electrolyte are then inserted in the internal feedback of the amplifier, so they may be amplified. If the output of the amplifier is connected to the top side of the capacitor, the signal may also be strong enough to affect the current flow in the electrolyte in undesirable ways.

Because of the possible interaction between the selected electrodes and the electrolyte, the parasitic coupling between the electrolyte and the top metal layer needs to be minimized particularly near the electrodes. Ideally, only the electrodes themselves use the top layer. If that is not possible, it is useful to consider the potential of the conductors. The optimal choice in the absence of parasitic couplings is the potential of the working electrode. Alternatively, a different constant voltage might be used. Both options ensure that the current flow resulting from the

measurement signal remains mostly unaffected, though the second choice may have a minor influence due to nonlinear interactions at the surface. The constant potential also shields the lower layers of the sensor from signals in the electrolyte. In order to maintain the constant potential, however, a low impedance source is required. This is a problem from the perspective of parasitic losses, since the low impedance does not impede current leakage. A solution would be to apply the driving signal to the top metal conductors. This is also an option for unselected electrodes if a defined potential is desired. However, this requires a distribution network for the signal and poses a risk of creating new parasitic couplings. For most components using the top metal layer, the actual choices for the potential are given by their connections. In the case of the compensation capacitors, none of these approaches is possible because both contacts of the capacitor are using signal levels. Nonetheless, it is useful to consider the implications of the available potentials.

In contrast to the couplings between the chip and the electrolyte, parasitic couplings between metal layers are not specific to biosensors. Because of their impact on chip performance, they have been studied extensively. Consequently, they are well modelled in design tools and accounted for in post-layout simulations. Effects of design decisions on the layout need to be considered early, though, to avoid time-consuming redesign cycles. The feedback with a large resistor chain in the feedback loop exhibits a large surface area subject to capacitive couplings, but since the parasitic losses are well modelled in the design environment, no major deviation between simulation and measurement is to be expected. T-structures as those formed by the resistors and the parasitic couplings have also been constructed using regular components to realize feedback impedances that would otherwise have been too large to be integrated. However, the large area also may lead to coupling of noise or other signals into the feedback path. Hence, the layout of these components is critical. TIA designs that do not use resistors avoid this problem, but would have to be investigated in detail if they provide better performance, since every component will exhibit parasitic couplings. One way in which alternative designs may help is by reducing the overall size of the circuit and exposing a smaller area. Besides this direct reduction of parasitic couplings, a compact design also allows fewer electrodes to share the amplifier. This means that fewer inputs need to be routed to the input, further reducing the components contributing to couplings at a very sensitive point. However, the second-generation biosensor already reduced the sharing to 16 electrodes, so the room for improvement is limited.

7.3. Summary and Conclusion

The second-generation biosensor performed well in the experiments, and in the dry tests, no weakness was observed within the scope of its specification. In spite of a lack of wet testing, it was possible to draw conclusions and valuable design lessons from analysis of the design and

the existing measurement results. While the design included considerable improvements compared to the previous generation biosensor, it did not provide significant advancement to the understanding of the underlying mechanisms of the parasitic effects. The improvements are due to good design practices, rather than explicit consideration of parasitic couplings. As these issues have long been recognised and modelled in circuit integration, standard design techniques have evolved to minimize their impact. Unfortunately, the interface to the electrolyte which is critical for biosensors is less well modelled, and it causes issues that are not present in regular integrated circuits. Accordingly, it is desirable to solve as many design issues on the circuit side in order to benefit from the advanced design environments.

As illustrated in Chapters 4 and 5, the first-generation biosensor has shown that parasitic couplings are a topic of increasing importance for large microelectrode arrays, and the second-generation biosensor improved on this. In particular, the amplifiers shared by only 16 electrodes avoided coupling the electrodes to long, common lines. It was also beneficial to minimize the exposure to parasitic coupling by reducing the active components and keeping the active circuits concentrated to avoid unnecessary stray coupling. The second-generation biosensors were an improvement in these respects, but a more dedicated approach has considerable promise for further enhancement.

A weakness of the design was a lack of driving strength, particularly for loads at the lower end of specification. Because the most relevant information for cell detection and characterization is found at relatively low impedance regions, it is important to address this weakness in future designs. A key observation was that the design of the second-generation biosensor was limited to one particular measurement topology. However, by adding a distribution network for an excitation signal and improving the driving capability of the amplifiers, the setup is extendable to an improved topology.

Chapter 8: Design of a 3rd Generation Improved CMOS Microelectrode Array Biosensor

8.1. System Level Considerations

A new CMOS array biosensor was designed to incorporate as many lessons from the previous designs as possible. As one of the lessons was that the common driving signal from the counter electrode led to problems with current leakage and parallel measurement, the EIS approach was reinvestigated. This investigation led to a number of different concepts, but only one biosensor design could eventually be realised within the scope of this Thesis. A high-level consideration for development was the measurement topology given by the placement of the driving source and measuring probe. This placement has consequences even in the absence of parasitic current losses in the device under test, as the probes themselves present a source of error. Voltage probes in parallel to the device allow a small amount of current to pass through them, whereas a voltage drop occurs over current probes in series to the device. Depending on the configuration of voltage and current probe, only one of the measured values accurately represents the value at the device under test. Current losses in the device may similarly affect the accuracy, even in the case of ideal instruments. To limit complexity, it is useful to abstract from the realization of the probe and source for analysis. Implementations of the measurement electronics may shroud the topology, though they retain the underlying behaviour. An example of this is the three-electrode setup for EIS measurement, which uses a controlled current source to realize a voltage source. From the topological perspective, this setup nonetheless behaves like a voltage source. In most common EIS systems, the source and probe sections are kept separate. This simplifies both design and analysis, but it is not necessary.

EIS measurement requires at least one source and one probe. One possible choice is to apply a voltage at one contact of the impedance under test Z_m and measure the resulting current at the other contact, as shown in Figure 8.1a. This configuration has the advantage that it is readily expanded to a four-electrode arrangement as in Figure 8.1b with two current bearing and two

Figure 8.1: Possible EIS measurement topologies: a) conventional voltage-driven current measurement; b) four-electrode setup; c) current-driven voltage measurement; d) one-sided measurement

sensing electrodes. This helps to eliminate the influence of leads to the impedance on the measurement results. Consequently, this is the most commonly offered configuration in commercial EIS systems. As explained in Chapter 3, however, the architecture underestimates the current flowing through the impedance if current leakage occurs. Accordingly, the impedance is overestimated. A topology with less clear separation of source and probe is needed if a current driven measurement is desired. This is illustrated in Figure 8.1c, where the current source and the voltage probe are connected at terminal 2. A fixed current is useful mostly if the device under test is well described by a series of impedances, as in that case the common current defines the state in each impedance. The voltage is measured across the device, so the accuracy of the results is limited only by the quality of the probe. However, leaked current does not contribute to the voltage drop beyond the point of leakage. Hence, the impedance is underestimated with this topology. For biosensors using EIS it is important to consider that the impedance shown in the figure is an idealization of the electrode/electrolyte system. This particularly affects the current driven topology, since in this case the voltage drop over the electrode/electrolyte interface is not limited. If the current becomes too large, it forces the interface into an operating range where additional electrochemical reactions may be driven, possibly damaging the electrode in the process. Even without substantially changing the electrochemical balance of the interface, the unconstrained voltage may lead to considerable non-linear behaviour of the electrode. Accordingly, the current driven approach is not a good choice for biosensors.

An unusual approach for impedance measurement is to place both the source and the probe on the same contact of the impedance under test, as shown in Figure 8.1d. In integrated form, this means that source and measurement are combined to a single source-measurement unit (SMU). This unit can also be implemented as a voltage source or a current source, though the latter is indistinguishable from the conventional architecture in terms of topology. Similar to the conventional measurement topologies, the voltage driven approach is usually preferable for biosensing applications since it ensures the voltages over the electrode/electrolyte interfaces are kept low enough to avoid non-linear behaviour or changing the electrochemical balance. The fixed setup of an SMU is less flexible than a setup using a separate source and probe. Because the SMU combines source and probe in overlapping circuits, the design may also lead to a loss of accuracy and precision. However, in an integrated biosensor the flexibility is limited by the hard-wired connections. The necessary accuracy and precision can be achieved within a range of impedance using good design practices, though the feasible range depends on the implementation.

Figure 8.2: SMU implementations: a) as TIA structure; b) as minimal structure; c) controlled current source

There are a number of ways to realize an SMU-based impedance measurement. The most straightforward implementation is illustrated in Figure 8.2a and structurally identical to a TIA based on an operational amplifier. The difference is in its operation, as instead of keeping a constant voltage at the differential input, the voltage is controlled by the interrogation signal at the positive input. The voltage at the negative input follows due to the feedback, and the output v_{out} of the amplifier is

$$v_{out} = v_{in} \left(\frac{Z_F}{Z_{mes}} + 1 \right) \quad (8.1),$$

where v_{in} is the input voltage, Z_F is the feedback impedance and Z_{mes} is the impedance to be measured. This approach is not suitable to measure large impedance values, as in this case the term depending on the impedance becomes negligible. However, the approach may still be used for biosensors, as the valuable information is obtained mostly in a frequency range where the total impedance is well below 1 M Ω .

A minimal implementation of an SMU-based EIS measurement topology is possible using only one transistor and a load resistance, as shown in Figure 8.2b. This approach uses the fact that the amplification of a common source amplifier depends on the load. By routing the measured impedance Z_{mes} in parallel to the load resistance R_{load} , with a transconductance g_m of the transistor M_1 , the amplification A becomes

$$A \approx -g_m \frac{Z_{mes} R_{load}}{Z_{mes} + R_{load}} \quad (8.2).$$

The load resistance would ideally be infinite to simplify the equation, but a finite value is necessary to prevent a DC current flowing through the sensor impedance. Due to the complicated response and the sensitivity to process variations, the circuit is not suitable for accurate measurements. Moreover, because the potential at the output is also the potential at the electrode, the voltage across the electrode/electrolyte interface may exceed the linear range. However, because of the simplicity of the circuit, it can be designed to operate at very high frequencies.

The more conventional implementation of the measurement topology in Figure 8.2c uses a differential amplifier to adjust a current source such that the voltage at the output is equal to the input. For an ideal amplifier, the required current is inverse proportional to the impedance. However, the current may not be straightforward to measure. It is possible to insert a resistor between current source and the feedback, but this affects the driving strength of the real current source and thus the frequency response of the setup. Alternatively, a copy of the current is needed to be measurable. The copy is not exact, though, leading to measurement errors. The errors are systematic, however, and do not affect the ability to detect changes of the observed impedance.

A benefit of the SMU-based EIS measurement is that the counter electrode can be kept at constant potential. This is particularly important if a large, shared counter electrode is used, as the large electrode and its supply lines present a considerable area to noise coupling and signal loss. The main advantages of the approach, however, stem from the possible concentration of the active elements near the selected electrode. This clustering means that the interrogation signal only needs to travel short distances between excitation and measurement. Besides limiting the potential for noise coupling, the short connections mean that there is negligible propagation delay for signals. Consequently, the maximum frequency for EIS measurements is only limited by the circuits. Current CMOS technologies provide transistors with intrinsic frequencies in the GHz range and dedicated technologies can operate up to hundreds of GHz.

Figure 8.3 shows a possible concept and implementation for a high frequency (HF) EIS measurement system. The design is based on the minimal SMU structure and extends it with a mixer and a low pass filter. The HF input is connected to the electrode and then mixed with the response from the electrode to obtain a low frequency (LF) representation of the phase and amplitude of the response. The low pass filter then removes remaining HF components that may

Figure 8.3: GHz capable EIS: a) concept; b) implementation

impede evaluation of the output. As the possible implementation in Figure 8.3b illustrates, few components need to operate in the HF range. Transistor M_1 and resistor R_{load} form the core amplifier controlling the input current of the measured impedance Z_{mes} . The ensuing voltage at point 2 is the response used as input for the mixer consisting of transistors M_2 , M_3 and M_4 . The low pass filter does not have to be built explicitly, as practical measurement circuits will have a low pass characteristic. Since only M_1 has to drive a considerable load, the area requirements are low, making integration with individual electrodes feasible. With the compact design and short propagation paths, operating EIS sensors in a frequency range relevant for molecular relaxation can be achieved without significant problems of signal reflection. This opens up an electrochemical sensing aspect beyond conventional EIS systems. Unfortunately, the design of the required high frequency circuits was not feasible within the scope of this Thesis. In particular, the distribution of the HF signal to the electrodes of a large array remains challenging. If the additional challenges can be met in a future project, the additional information from higher frequency ranges may, however, well justify the effort.

For EIS measurement systems using frequencies below 100 MHz, the high frequency effects are negligible. A more relevant advantage of the SMU approach is that the measured current includes all leakage current. Although the combination of driving and measurement circuit may negatively affect the accuracy of the result, these effects are modelled in the design environment and can be accounted for. Moreover, the detection and analysis capability of the sensor is not dependent on the absolute value of the measurements, as only relative changes of the measured impedance contain the relevant information. An advantage of using the working electrode as driving electrode is that it ensures that the highest current concentration and hence the highest sensitivity to changes occurs at the electrode. Because the total current is measured, this effect remains valid in spite of current losses from the electrolyte due to parasitic couplings. Since the current intensity decreases with the distance from the electrode, the impact of the parasitic coupling also falls with the distance. Hence, the total impact is reduced compared to setups driving from the common counter electrode.

8.2. Design Specifications and Goals

A starting point for the development of the new design was the second-generation biosensor, and the initial specifications for the new design, listed in Table 8.1, have been adapted from this earlier circuit. This was meant to add meaning to the obtained results, as it provides a frame of reference for comparison. However, the goals of the new design are very different from the previous generation CMOS array, as the main objective is to show the viability of integrated EIS sensing with a SMU topology. Consequently, instead of a large integrated array, the design comprised only an electrode field of 8×2 electrodes. Instead of preserving the electrode density, the electrode pitch was increased to 60 μm . The size of the electrodes was kept identical, though,

Parameters	Value			Unit
	Min	Typ	Max	
Electrode Count		16		
Electrode Size (Al)	17		19	μm
Electrode Pitch		60		μm
Supply Voltage		3.3		V
Excitation Voltage	10	25	50	mV
Impedance Range	1		1000	k Ω
Current Range	1		1000	nA
Frequency Range	1		10000	kHz

Table 8.1: Specification of the proof of concept biosensor

for easy comparison of the results in wet tests with a pixel structure from the second-generation biosensor with 4×4 electrodes that was also included on the chip. Another important novel aspect in the design of the new biosensor was the system architecture to support SMU-based EIS measurement. Accordingly, the implementation of individual system components needed to be relatively straightforward to avoid surprising interactions between modules. There were considerable requirements on the individual components to achieve accurate and stable signal levels, though, particularly the potential at the electrode which needs to be well defined. Moreover, it is desirable to avoid uncertainties due to process variation. Consequently, the architecture of the amplifiers could not be simplified significantly to reduce complexity or area requirements. However, the need to accurately set the voltage originates from the requirement for absolute measurements, which for the biosensor results from the need to provide comparable measurements. This need only exists during the assessment of the sensing approach, as the information is contained in relative changes of the impedance. Hence, future biosensors may be able to use significantly less complex circuits for sensing, which is important if the application requires to increase the electrode density or reduce the number of electrodes sharing a single input amplifier.

Since the measurement target for the new biosensor is the same as for previous designs and the electrodes have the same geometry, the operating parameters required only minor review. The exception to this was the expected impedance range. Since the impedance was overestimated by the previous biosensors, the minimum impedance for the measurement was reduced to 1 k Ω . Such an impedance presents a considerable load for the integrated driver in spite of the low output swing used for EIS measurements. In fact, the small offset to the operating point contributes to the problem in that the bulk of the voltage drop and consequently heating occurs within the driving transistors at all times. Though the required current is very low due to the small signal voltage, permanently driving this current is not desirable in the light of parallel measurement at several electrodes. The peak amplitude of the signal was specified as 50 mV, since this was found to be the maximum voltage over the electrode/electrolyte interface without considerable non-linearity [51]. This signal amplitude alone would lead to a maximum

current of 50 μA into the load, but with safety margins to ensure linearity of the output a significantly larger current may be needed in the driving path for a class-A output stage as it was used in the second-generation biosensor. Consequently, the output stages of the amplifiers used in the new design required considerable effort. Similar to the operating voltage, the frequency range of the EIS measurement is given by the electrode/electrolyte system under test and hence essentially the same as in the previous designs. However, to avoid the need to balance out the frequency response of all stages of the pipeline, the cutoff frequency was defined only for individual components. As there is no external feedback to the signal chain, no stability issues are introduced by the connection of the modules. Since the most relevant changes in the measurement response for cell detection have been found in the region around 100 kHz [33], the significant frequency range extends from 10 kHz to 1 MHz. For the specification of the biosensor modules, the range has been extended by a decade in both directions to minimize the distortions within the critical range. This is particularly important since the individual modules may have similar cutoff frequencies and the drop-in amplification accumulates.

8.3. *Sensor Architecture Overview*

Similar to TO2, the new biosensor comprises only the core interrogation unit, rather than implementing the entire sensing chain. To complete an EIS measurement system, the interrogation signal needs to be generated and evaluated externally. Besides reducing the development effort for the design, this also allows for flexibility in the choice of interrogation signal. The counter electrode is also a necessary external component to the biosensor in wet tests. For tests of the SMU-based measurement, the counter electrode needs to be connected to common mode potential. For comparative measurements in conventional topology, the counter electrode is driving the interrogation signal. The connection between counter electrode and the biosensor is given by the electrolyte solution. In dry tests, the counter electrode and electrolyte are replaced with a test cell and probe, but the principle remains the same. A microcontroller is used to configure the chip, though in principle the interface is simple enough to make the settings with manual switches.

Figure 8.4: System overview for the proof of concept biosensor

As illustrated in Figure 8.4, the chip internally comprises two major blocks, one comprising the new design and the other consisting of a pixel structure from the second-generation biosensor. As the pixel structure serves for comparison, it is left unchanged from the original design, though the surrounding support circuits are simplified. Addressing of both designs is done through a shared logic block. This logic block is mostly incorporated in the new design, as it had to be newly created. In particular, the addressing logic is partially integrated with power management functions.

The main components of the new design, illustrated in Figure 8.5, are the signal distribution and the electrode array. As the distribution network to the electrodes presents a considerable load, a signal buffer is used at the input to the chip to ensure distribution of the signal without significant distortions. The large network also presents a considerable surface for parasitic coupling, leading to the danger of coupling the signal into the electrolyte or into a wire on the measurement side, both of which would distort measurement. Moreover, driving the signal leads to a drain on the supply. This in turn leads to fluctuations on the supply lines which also disturb the measurement. Because both the signal distribution and the load balancing are important features of the design, the output was made available for testing through a contact pad. The available output at this pad is selected through transmission gates, using the same output that is also used by the selected electrode. The electrode array is arranged in a field of 2×8 electrodes, which allows the area of the interrogation circuit to grow as needed. The focus of the development of the electrode array was to demonstrate the SMU topology, since it represented

Figure 8.5: Block diagram of proof of concept biosensor

the original part of the design. As a consequence, there is no sharing of circuits between electrodes and every electrode has its own dedicated interrogation unit to avoid unnecessary complications to the design. Similarly, the interrogation signal flows in a single direction from the source to the sensor output to avoid unnecessary interaction between modules.

8.4. Circuit Design and Layout

Individual blocks were designed to be functional, rather than optimal. Consequently, individual circuits were largely taken from the literature as is, and component parameters were merely adjusted in simulations. As the design does not require much configuration, it contains few logic components. Most settings are made directly from input signals. The remaining settings are made using asynchronous CMOS logic. The only major logic component is the address decoder, which is shared between the old and new design blocks. Because there is no clock signal, a separate enable signal is used to activate the selected electrode to avoid glitches. Besides controlling the power supply of the driving circuit and the coupling to the electrode, the selection also manages the bias current supply. To facilitate this, the bias current is distributed in a tree of switchable current mirrors. The source of the bias current is a self-biasing circuit using an external resistor for adjustment.

The signal buffer, illustrated in Figure 8.6, is part of the input interface of the design and is necessary to provide the driving strength to supply the interrogation signal network with a stable input. This network may present a considerable load due to its size, and the load is hard to predict until post layout simulations are done. Consequently, the most important part of the module are the load buffers at its output. Two buffers are needed, since there is a positive and a negative copy of the interrogation signal. Each buffer is an operational amplifier in unity gain configuration, based on a circuit described in [84]. A schematic view of an output load buffer is shown in Figure 8.7. The amplifiers use an NMOS differential input with a folded cascade for

Figure 8.6: Block diagram of signal buffer

Figure 8.7: Schematic view of load buffer

improved gain. An auxiliary amplifier is used to improve the frequency response. Moreover, an AB-mesh is integrated with the folded cascode to provide the input for a push-pull general amplifier output stage. Because the load buffers provide a high driving strength and a wide input and output range, they have also been reused as output buffers of the chip.

The input of the load buffers is provided from the differential copy generator. The purpose of this unit is the creation of a direct and an inverted copy of the interrogation signal to balance the effects of the driving currents on the power supply and coupling into other conductors. The two signal copies are generated mostly independently in two buffers from a differential input, though the bias generation and common mode feedback are shared. The design is shown in Figure 8.8. The input signal occupies both inputs of a differential pair, so a second differential pair was added to enable feedback. This architecture avoids on-chip resistors, which would present a large area to couple in noise. A folded cascode is used to increase the open loop amplification, but no additional driving stage is included since the buffer only needs to drive the separate load buffers. The combination of the two modules into a single, fully differential load buffer was investigated as well. Besides reducing the area requirements, this design also reduced

Figure 8.8: Schematic view of differential copy generator

the current consumption. However, the frequency response of the split design was easier to stabilize, so the combined buffer design was discarded.

Figure 8.9 shows the differential copy generator. The input of the differential copy generator can be switched using transmission gates between two sources. In one mode, the input is driven directly from an off-chip source. This is mostly meant for analogue sources like function generators or commercial EIS instruments. The alternative mode is using a digital input and scales it from a regular full swing signal to the desired low amplitude used for EIS. This is needed because it is impractical to distribute or generate digital signals directly at the lower amplitude. In particular, the buffer was included in the design to allow testing with digital signals generated with a microcontroller. The scaling is achieved by switching one side of a differential amplifier between two input paths. The path activated by a logical zero uses the common mode voltage as input, whereas the path activated by a logical one uses a reference voltage as input which differs from the common mode voltage by the desired amplitude. Besides the switched input, the unit is an operational amplifier in unity gain configuration. A folded cascode is used

Figure 8.9: Schematic view of signal switch

as output stage to boost the open loop amplification without compromising the bandwidth of the amplifier.

The center of the measurement system is the SMU interrogation module. In principle, this unit is a TIA, with the only exception that the voltage at the test point is varied instead of being kept at ground potential. Internally, the TIA consists of a driving amplifier in unity gain configuration, shown in Figure 8.10, and a current mode measurement amplifier, shown in Figure 8.11. As the driving current of the DA is used to drive the electrode, a copy of the current is needed as input to the measurement amplifier. The copied current is scaled down by a factor of 10 to avoid an unnecessarily large input range for the measurement amplifier. The driving amplifier was originally adapted from the load buffer, since the two modules face similar issues. However, to facilitate decoupling from the electrode and add a safeguard against potentially harmful output voltages, the output stage was changed to a source follower configuration. Consequently, the folded cascode stage with auxiliary amplifier had to be adapted. The source

Figure 8.10: Schematic view of electrode driver circuit

Figure 8.11: Schematic view of measurement amplifier

follower configuration eliminated the need for the AB-mesh, but the input range prevented the use of a cascode in the pull-down path. The measurement amplifier was developed based on a circuit presented in [85]. The design had the advantage of using few components, but this also made it difficult to balance the linearity and current consumption. It was straightforward to build a high gain or a low gain version of the circuit, but because amplification was effectively determined by the bias current and the configuration of M_{13} and M_{14} , no compromise solution was found within the available time frame. A complete redesign using a different circuit was considered, but also rejected due to a lack of time. As the high amplification version could not be used for the highest possible input currents, the low amplification was chosen. The output of the measurement amplifier is differential, helping to protect the small output signal from disturbances.

A completely differential design of the interrogation module was contemplated as well, since internally both the driving and measurement amplifier are already constructed mostly differential. However, as there is no differential equivalent of the electrode, the measurement would have reflected an imbalance in the amplifier. Though this is a valid option, it would have introduced another unestablished element in the system. Moreover, the differential design would have required a second feedback capacitor. Consequently, whereas the fully differential design is interesting for future biosensors, it has been rejected for the current design.

Due to delays during circuit design, the layout of the biosensor had to be done in a short time. As a result, the layout could not be optimized beyond standard design techniques. Neither the required chip area nor the parasitic coupling were minimized exhaustively. The TIA occupied an area of $600\text{ }\mu\text{m}$ by $60\text{ }\mu\text{m}$, which is too large for later integration in a high-density microelectrode array. However, a considerable part of the area is required for long channel transistors used in current mirrors to ensure a good match between currents. The complexity of the amplifiers also contributed to the issue. Both factors may be avoided in future designs through the use of simplified amplifier architectures at the cost of reduced open loop gain. This loss may be partially compensated through circuit optimization. Especially the channel length of transistors was chosen to be $1\text{ }\mu\text{m}$ following experience with previous designs, rather than minimized. A remaining, minor loss of signal amplitude is expected to be tolerable, as it can be taken into account in the measurement.

A minor design optimization that was integrated within the available time frame concerned packaging. In the first and second generation biosensors, the connection to the counter-electrode was considered after the sensors had been fabricated and tested. As a consequence, the contact was not straightforward to create. The new biosensor features a pad which is connected directly to another surface contact at a safe distance of 1 mm from the bond pads and the electrodes. This way, a conductive adhesive can be placed on this contact to facilitate the connection to the counter electrode.

The biosensor that has been developed for this Thesis is meant as a first step towards SMU-based EIS measurement in microelectrode arrays. Many design decisions will have to be reviewed in future implementations, and some have already been identified as problematic. Nonetheless, the design is a faithful implementation of the underlying measurement approach. As such, it provides an important milestone on the way to promote the SMU-based EIS measurement with significant potential for microelectrode arrays employing EIS as sensing mechanism. Although a signal source and voltage probe are required externally to complete the measurement setup, the design provides the core that differentiates the SMU-based measurement from more conventional measurement topologies. As the remaining external components are common to EIS measurement systems, it is this core that needs to be demonstrated as a suitable alternative. Although the individual modules have room for improvement, the developed structure is adapted to this task.

The new biosensor was manufactured in the same 0.18 μm Global Foundries standard CMOS technology that had also been used for the second-generation biosensor. The post-processing steps remained unchanged as well. The changed surface topology may have had an effect on the material deposition, but its variations were too small to significantly change the surface properties. Before manufacturing, the design was thoroughly investigated in simulations. Besides being a necessary step to avoid the costly fabrication of a defective design, the simulation already provided support to the viability of the measurement approach. As the investigations on the second-generation biosensor have shown, for dry tests, a good match between simulation and manufacturing is expected. The parasitic effects that are added by the common electrolyte are related in scale to the size of the array and accordingly unlikely to show strongly in the 2×8 array of the new design.

8.5. Simulation Results

The simulations were done using Cadence® Virtuoso® Spectre® Circuit Simulator as part of the design process. A significant part of verification was accomplished already while optimizing the frequency response of the circuits, since stability was a major design goal. Final simulations were only possible after completion of the layout, however. A key difference between simulations during optimization and simulations for verification was that only typical transistor parameters were used during optimization, whereas verification required mapping the spectrum of varying transistor parameters and identifying the worst case behaviour. Module testing began with simulation of the steady state behaviour to monitor the set point and operating range of the circuit. This was followed by frequency domain small signal simulations. Amplifiers were mostly simulated in open loop configuration, as this was needed for evaluation of amplifier stability and open loop gain. Only once this was settled, closed loop simulations were done to observe the expected behaviour in the actual configuration. Finally, transient

simulations were done to double check stability and investigate issues which are not visible in small signal behaviour, such as linearity.

The signal buffer was verified both in separate submodules and in its completed state. The individual components were mostly tested in the frequency domain, whereas the completed buffer was also verified in transient simulations. A special case was the scaling unit for digital input, as there was no true input node operating in small signal mode and the frequency was tested only for stability. A load of 10 pF was chosen as an estimate of the load, since the network presents a considerable but not too large load due to the relatively small electrode array. The obtained frequency response shown in Figure 8.12. Both the positive and the negative copy of the signal dropped by less than 3 dB at 10 MHz. A rapid drop in amplification appears around

Figure 8.12: Signal buffer frequency response

Figure 8.13: Transient response of signal buffer

30 MHz, but this is a result of the individual buffer stages having similar cutoff frequencies. As there is no feedback between these stages, this is actually beneficial, since it helps to suppress undesired high frequency components of the signal. Beginning from 10 kHz, a slight divergence has been observed between the two signal copies and traced back to a difference in common mode rejection. Although this is disadvantageous for the balanced use of the two copies, it becomes critical only beyond the specified frequency range. Up to 50 MHz, the difference remains below 1 dB. Transient mode simulations for the signal buffer were exclusively done in digital stimulus mode, as it covers the entire circuit and provides sharp signal edges. In particular, this mode allows observation of high frequency components which are introduced in the scaling unit through capacitive coupling and attenuated in the further buffer components. The frequency of the input signal was varied between 100 kHz and 1 MHz to observe the behaviour of the buffer under the different conditions. The rise and fall times were kept at 5% of the period, and the duty cycle was kept at 50%. The buffer introduced minor distortions, with the high level followed better than the low level, as can be seen in Figure 8.13. The distortions were predominantly caused by the scaling unit and were accepted in the design because the digital stimulus was a secondary function of the design. Moreover, the relatively slow trailing of the signal helps to filter out the high frequency components from the capacitive coupling at the input.

The driving and measurement amplifier of the interrogation unit have been verified in separate simulations, since the signal is flowing in one direction with minimal feedback. The main issue for the driving amplifier is stability rather than driving strength. The dominant poles of the frequency response result from the folded cascode stage, since a relatively weak driving strength has to charge the Miller compensation capacitor. Consequently, the amplifier was mostly tested with a moderate load of a 10 pF capacitor, rather than the maximum load of 1 k Ω . The frequency response of the driving amplifier for this load is given in Figure 8.14. The circuit has less than 2 dB loss of amplification at 10 MHz. A steep decline occurs shortly after, but since these characteristics are due to the folded cascode and the chosen compensation scheme, the behaviour does not change significantly up to the maximum load. The measurement amplifier operates as a TIA, hence in simulations it was excited by a current source and provided a voltage signal. As the output is of a different type than the input, it is potentially misleading to use the common, dimensionless, double logarithmic view of the frequency response. The amplifier also comprises only a single stage, so the frequency response, shown in Figure 8.15, is straightforward. Consequently, the output of the module was examined in linear scale for most simulations. The output signal of the amplifier is small, but since amplification can be done in later stages or even off chip, this is not a major concern for the proof of concept. A more importantly property of the module is that the amplifier remains linear for the required operating

Figure 8.14: Electrode driver frequency response

Figure 8.15: Measurement amplifier frequency response

range and does not saturate. Another positive aspect of the design is that the cutoff frequency occurs beyond 100 MHz, well outside the operating range.

Transient simulations were mostly done after the interrogation cell was completed, since they were meant primarily as confirmation of the results from the frequency response and did not provide much additional information for circuit optimization. The majority of simulations used an input signal with a frequency of 500 kHz. This frequency has been chosen as a compromise between the most relevant signal range, assumed to be around 100 kHz, and the cutoff frequency of the module, which dictates how fast the system is able to follow the signal. The rise and fall times of the input signal have been chosen as 100 ns, and a duty cycle of 50% was used. For the load at the electrode, the test cell shown in Figure 8.16b was used. This test cell had also been used in previous simulations of the second-generation biosensor. Though this may not reflect the actual impedance at the electrode, it is expected to be uncritical for the

Figure 8.16: a) Transient response of the SMU core; b) used test cell

stability of the interrogation unit. The transient response of the SMU core unit is given in Figure 8.16a. The signal at the electrode follows the input signal very closely. As the Measurement Amplifier receives the driving current as input signal, the response appears only at the signal edges, where the strong peaks reflect the charging of the capacitance at the output, whereas the small deviations from zero reflect the current through the 100 kΩ resistor.

The purpose of the simulations was to validate the chip before production. In order to do this, the results needed to show that the individual components and the design as a whole function for the expected variation in the quality of circuit elements. In particular, it is important to show that the system is capable of measuring an impedance at the electrode. Detailed properties such as linearity of the measurement or a good output range are not a significant concern, since the design is merely a proof of concept. As the chip comprises the core interrogation unit rather than the whole measurement system, it is sufficient to show that the design reacts to a change of impedance in a deterministic manner. The support circuits of the biosensor were straightforward to verify, with the exception of the bias generator. For all tested parameters, the bias generator successfully started up as well and delivered the desired bias current. The core of the design consists of amplifiers and buffers, and the most critical properties that needed to be shown are stability and amplification. Both of these are related to the frequency response of the modules, and the observed responses are essentially constant until the cutoff

frequency. The results have been confirmed in the transient analyses, which showed that there was no noticeable instability. Hence, the simulation results show that the individual modules of the design are working within the specification. The interrogation unit has also exhibited a reaction to the impedance at the sensing input. The output signal was small but roughly proportional to the admittance, though this has not been quantified as it went beyond the objective of the design. Since the sensor is meant as demonstration of the concept, this is a satisfactory result. A drawback observed in the transient simulations with digital stimulus is that the dominant reaction occurs very shortly after the signal switch. The strongest peak occurs due to the interrogation unit quickly charging the capacitive components at the electrode. As a consequence, it is very hard to capture the response with sufficient precision to extract the relevant information about the load on the electrode. Since linearity is preserved, it may still be possible to analyse the signal, though it would likely require considerable filtering or a significantly higher base frequency of the system to obtain a usable response.

Unfortunately, measurement results have not been able to confirm the simulations yet. Because the test platform became available late, experiments could only be performed on a single chip. In these experiments, the chip output appeared to be stuck at maximum output voltage. The reason for this behaviour remains undetermined at the time of writing. It is not certain if the source of the problem is on-chip or off chip, though errors have been found and corrected on the test board. Since the chip did not possess a protective glob top cover because the electrodes had to be accessible, the error may also have been caused by touching bond wires. Within the chip, the most likely error source is the start-up circuit of the bias generator failed. The only alternative source is the signal buffer, as no signal was found at the test output of the buffer. However, because the error found is inconsistent with any simulation results and only one chip has been tested, it has not been possible to identify the bias generator as the source of the issue with certainty. As the output of the chip is stuck at the supply rail, the core function of the chip cannot be monitored, and it is impossible to judge it from the results. Whereas the error needs to be identified before future attempts to develop an improved chip, this appears to be unrelated to the SMU-based EIS measurement. However, the simulation results already provide support for the concept. As the studies on the second-generation biosensor have shown, a good match between simulation and experimental data would be expected in dry tests.

Within simulations, all components complied with the specification, and the impedance measurement is an emergent behaviour of those components. Though experimental results are still needed as proof of concept, the approach is expected to work well and the failure of the design is surprising. The lack of experimental results is an incentive rather than a deterrent for further investigation. The SMU approach offers considerable promise to improve EIS measurement on microelectrode arrays, particularly with respect to parasitic couplings. The approach has even more potential if used with high frequency measurement or improved

electrode structures. Hence, though the method could not be demonstrated yet, the concept is worthy of further investigation.

The electrodes forming the connection to the electrolyte are a central part of biosensors. The interface characteristics are complex, and the parasitic connection may have a significant impact on measurement, as shown in Chapter 5. Consequently, the electrodes cannot be regarded as a mere contact point, and the construction of the contact deserves considerable attention. Due to the impact of the electrodes on the measurement, their design is relevant regardless of the topology used. The use of SMU-based measurement also has the potential to change the impact of the electrodes, giving merit to further analysis.

An important aspect of this design is the geometry of the electrode itself. The choice of geometry offers considerable room for optimization, especially for increasing the electrode surface. However, the options are limited by manufacturing constraints and the available area for the electrode. Additional flexibility can be gained by employing multiple electrodes in close proximity. The potency of this approach is illustrated by interdigitated electrodes, which facilitate keeping the sensing field concentrated closely above the electrodes. Because source and sink are in close proximity, interdigitated electrodes benefit less from SMU-based measurement. They do, however, require a similar infrastructure, as the interrogation signal needs to be distributed as well. Depending on how the distribution is accomplished, arrays of interdigitated electrodes may also suffer from considerable parasitic coupling, which is particularly critical because of the large impedance of both the driving and the sensing electrode. An efficient method of signal distribution for arrays of interdigitated electrodes is to drive a row of sensing sites at a time, while reading out the response in columns. The main advantage of this approach is that it facilitates a highly parallel measurement. The layout is also highly regular, reducing the design effort. These regular structures increase the danger of crosstalk between neighbouring lines, though. As the structures stretch across the entire width or height of the array, there is also a large surface area prone to parasitic coupling. However, the impact of the coupling is limited due to the close proximity of the interdigitated electrodes, binding the field lines close to the surface of the array.

Another form of using multiple electrodes in close proximity to improve the electrode behaviour is given in three and four electrode setups. In a conventional EIS architecture, three electrode setups are used to reduce the impact of the driving electrode on measurement. For this purpose, the driving electrode is split into the actual driving electrode and a current free sensing electrode. The sensing electrode is then used to adjust the current until a set potential is reached. Because no current flows into the sensing electrode, the measured potential is identical to the potential in the electrolyte.

If a suitable TIA design is used, the extension of an EIS measurement system to a three-electrode setup is straightforward. A common design approach for a TIA is to use an operational

amplifier to adjust a current source acting as sink for the input current. Instead of connecting the feedback current directly to the amplifier input, the injection point is in a first step moved to the electrode. The electrode is then divided into two parts. The segment connected to the feedback becomes the driving electrode, whereas the segment connected to the amplifier input becomes the current free sensing electrode. The inner architecture of the amplifier is not directly affected by the changed setup, since the load remains largely unchanged. However, the control of the current through the sensing electrode must be ensured to avoid a potentially damaging fault state. The modification of the TIA moreover requires that the feedback current does not depend on the potential at the electrode, because after the separation a potential difference exists between the driving and the sensing electrode. Accordingly, the TIA design used in the second-generation biosensor is not suitable for the modification, since the current is derived from the feedback resistor and the voltage between output and input. The design used in the improved sensor described in Section 7.1 could be utilised in a three-electrode setup. As illustrated in Figure 8.17, the only necessary modification of the electrode driver from Figure 8.10 is a separation between the feedback input from the sensing electrode from the driving electrode.

In principle, the three-electrode approach works independently of the potential set point, although circuit limitations and constraints from electrochemical reactions still apply. Accordingly, it is possible to implement both conventional architecture or SMU-based measurement with a split working electrode setup. Another consequence is that the setup can be further extended to a four-electrode setup, using two electrodes each at both the current source and the current sink of the measurement system. This way, the potential is fixed close to both sensing electrodes. With the voltage between two points within the electrolyte fixed, the current between them is determined only by the impedance between those points. For an EIS biosensor,

Figure 8.17: Schematic view of electrode driver modified for split electrode measurement

this impedance is dominated by the characteristics of the electrolyte and the current blocking cells. Hence, the influence of the cells on the impedance that is measured is much larger now compared to the more basic two-electrode setup.

However, it is important to note that the utilized control scheme only aims to fix the potential near the sensing electrode. The potential near the driving electrode may be arbitrarily different if the distance between the electrodes is large. To set a bulk potential in the electrolyte, it is sufficient for the sensing electrode to be close to a large, common driving electrode and far from the working electrode, since this setup provides a similar potential over a large area. In the case of a split working electrode, however, the steep gradients in the vicinity of the small driving electrode lead to a significantly different potential at the sensing electrode. Consequently, the placement of the sensing electrode needs to be considered carefully.

The potential difference between the sensing and the driving electrode is increased if the electrodes are made from different materials. In this case, an electrochemical cell is formed, producing a constant offset in voltage. This is largely irrelevant for measurement, since the difference originates in different electrode potentials and the potential difference within the electrolyte is unaffected. No constant current flows into the sensing electrode, and no higher frequency components are generated by the electrochemical cell. However, the effect needs to be considered with respect to the operating range of the driving circuits.

More relevant for measurement is that the presence of parasitic couplings violates the assumption of current free sensing electrodes. Current leaks from the conductors between the electrode and the amplifier, and the amplifier input also requires an input current. If the sensing electrode is not close to the driving electrode, parasitic current losses contribute to the potential difference between the driving and the sensing electrode. If, however, the driving and the sensing electrodes are close, the signal couples directly from the driving contact into the sensing contact. The effects are opposite in nature, so it is possible to balance the net influence on potential control, but this is limited by manufacturing variations and differences in frequency response.

For analysis of the setup, fixed potentials are assumed at the counter electrode and the sensing electrode. The potential at the counter electrode V_C is fixed externally, whereas the potential at the sensing electrode is regulated by the control scheme to match a given set point V_W . The impedance Z_{mes} is measured via the current from the driving electrode I_W according to:

$$Z_{mes} = \frac{V_C - V_W}{I_W} \quad (8.3).$$

Figure 8.18: Split electrode setup: a) without parasitic coupling; b) with parasitic coupling

The voltage between the sensing and the driving electrodes becomes relevant only if the placement of the sensing electrode leads to insufficient coupling. Otherwise, the potential of both electrodes will be on the same order. Since EIS utilizes low amplitude signals, the difference has then no impact on circuit design. If the counter-electrode is assumed to be ideal, it is then sufficient for a basic analysis to approximate the setup with three resistors as in Figure 8.18a. The resistor R_{CD} represents the direct path between the counter electrode and the driving electrode, which form the source and sink of all current in the system. Part of the current flows by the sensing electrode, determining the measured potential. This second path is represented by a series of two resistors, one resistor R_{CS} between the counter electrode and the sensing electrode, and another resistor R_{SD} between the sensing electrode and the driving electrode. The current I_{CS} flowing through R_{CS} is given by:

$$I_{CS} = \frac{V_C - V_W}{R_{CS}} \quad (8.4).$$

As the sensing electrode is current-free, the same current has to flow through resistor R_{SD} , which is the case for a potential at the driving electrode V_D of

$$V_D = V_W \left(1 + \frac{R_{SD}}{R_{CS}} \right) - V_C \frac{R_{SD}}{R_{CS}} \quad (8.5).$$

The current I_{CD} through resistor R_{CD} is then

$$I_{CD} = (V_C - V_W) \frac{1 + \frac{R_{SD}}{R_{CS}}}{R_{CD}} \quad (8.6),$$

and the total measured current I_W is:

$$I_W = I_{CD} + I_{CS} = (V_C - V_W) \frac{R_{CS} + R_{SD} + R_{CD}}{R_{CS}R_{CD}} \quad (8.7).$$

Consequently, the measured impedance becomes:

$$Z_{mes} = \frac{R_{CS}R_{CD}}{R_{CS} + R_{SD} + R_{CD}} \quad (8.8).$$

The true impedance between the counter electrode and the driving electrode Z_{real} , however, is given by:

$$Z_{real} = \frac{(R_{CS} + R_{SD})R_{CD}}{R_{CS} + R_{SD} + R_{CD}} \quad (8.9).$$

Hence, the larger the impedance is between the driving electrode and the sensing electrode, the more the split electrode approach underestimates the true impedance.

For the presented case without parasitic couplings, the estimate is not affected by the specific potential chosen for V_C and V_W , and only their difference determines the current in the setup. Consequently, the estimate is not affected by the measurement topology, since the voltage amplitude between the sensing electrode and the counter-electrode is not changed. However, parasitic couplings affect the topologies differently, similar to the two-electrode setup analysed in Chapter 4. To account for parasitic losses, in Figure 8.18b resistor R_{CS} is split into R_{CA} and R_{AS} , and resistor R_{CD} is split into R_{CB} and R_{BD} . Capacitive loss paths C_A and C_B to ground are added between the partial resistors. The path between driving electrode and sensing electrode is too short to produce significant losses, but the resistor may be bypassed by direct capacitive coupling through C_{SD} . The T-circuit consisting of R_{CA} , R_{AS} and C_A is of particular interest because it affects the control loop. Due to the current being lost through C_A , the current I_{AS} flowing through R_{AS} depends on the individual potentials V_W and V_C :

$$I_{AS} = \frac{V_C - (1 + j\omega C_A R_{CA})V_W}{R_{AS} + R_{CA} + j\omega C_A R_{AS} R_{CA}} \quad (8.10).$$

Consequently, depending if V_W or V_C are used to introduce the interrogation signal, the frequency response of the current will change. For low frequencies, the current is determined by the difference between V_C and V_W , since the capacitive losses are negligible. However, for high frequencies, the influence of V_W dominates, which means that the current and hence the control feedback is determined by the potential forced at the sensing electrode. In case of a conventional measurement topology, this potential is equal to ground, so the current is expected to become negligible. This implies that there is also negligible current between the driving electrode and the sensing electrode. In the presence of noise, this becomes problematic, since a small disturbance can dominate the feedback of the amplifier. In contrast, for measurement with a SMU topology, the current I_{AS} is larger for higher frequencies than for lower frequencies. This reflects the feature that the parasitic couplings bypass the part of the impedance represented by R_{CA} .

The observed behaviour is hence similar to the two-electrode setup considered in Chapter 4. The feedback loop may emphasize the effect slightly, but the amplification does not qualitatively change the feasibility of measurement topologies. The frequency from the analytic

model response becomes more convoluted, though this is mostly explained by the complexity of the model. Nonetheless, the model cannot accurately represent the frequency response due to the distributed nature of the parasitic coupling and uncertain properties of the individual components. Consequently, as in the case of the two-electrode setup, simulations are needed to study the behaviour in more detail.

8.6. Improved Electrode Configurations for EIS Array Measurements

The setup used for simulations exploited rotational symmetry with a sensing electrode at the centre and a driving electrode around it. The radius of the sensing electrode was $3\text{ }\mu\text{m}$, whereas the driving electrode had an inner radius of $5\text{ }\mu\text{m}$ and an outer radius of $15\text{ }\mu\text{m}$. Surrounding the driving electrode was a passive electrode, meant to simulate inactive electrodes in an electrode array. Simulations were done for the case of a static equilibrium, because this represents the highest impedance of the electrode/electrolyte interface and hence the worst case for coupling between driving and sensing electrode. The results found for the current flow and potential distribution in the electrolyte are illustrated in Figure 8.19a. The potential distribution, indicated by the solid lines representing equipotential surfaces, approaches the distribution of a single electrode with distance from the electrode. However, close to the electrode the field shows significant distortions. Consequently, the current flow, illustrated by the dashed lines is also changed. Close to the surface, the current spreads inwards to fill the space above the sensing electrode.

If a cell is settled on the electrode, the inward current may partially be blocked by the cell. In contrast to measurement using a two-electrode setup, this is not desirable, because the feedback loop is affected. If the feedback is inhibited, the current supplied by the driving electrode may increase to compensate instead of being reduced by the obstructing cell. This can be avoided by placing the sensing electrode as a ring around the driving electrode instead. However, the ring structure increases the area requirement for the sensing electrode, since the minimum structure dimension remains unchanged. The total area available for the electrode is

Figure 8.19: Simulation results for split electrode setup: a) potential distribution and current flow; b) electrode potential for given set point

limited, so the driving electrode has to be reduced in size. This presents a problem for the split electrode approach, because the area of the driving electrode determines the voltage drop over the interface necessary to reach a desired potential in the electrolyte. Consequently, a smaller driving electrode leads to a larger required driving strength. The impact of the cell blocking the inward current is furthermore small, because the sensing electrode is meant to take up little current anyway. Hence, the inner sensing electrode remains the preferable option.

Figure 8.19b shows the potential at the driving electrode required to force a given target potential for the setup using a sensing electrode at the centre. Since the control loop is not assumed to be perfect, the potential at the sensing electrode does not meet the target potential exactly, though the difference appears negligible for the investigated potential range. Nonetheless, the difference needs to be investigated during an actual implementation. The required potential at the driving electrode is an order of magnitude larger than the set point in spite of the close proximity between the electrodes. The driving voltage is limited by destructive processes at the electrode, though, so the target potential may not be attainable in the electrolyte, even if it is feasible for the driving circuit to achieve. The magnitude of the difference is also large enough to have an impact on circuit design. In particular, the offset voltage of the amplifier becomes a major consideration, since a substantial output offset is required to adjust for even minor input offset. Moreover, additional safety measures need to be integrated to avoid damaging magnitudes of the output voltage. It should be noted, though, that the necessary signal amplitude is reduced by the split electrode approach, since the potential is forced directly in the electrolyte and the losses over the interface are compensated by the control loop. Consequently, the effective signal amplitude is significantly higher than the set amplitude of the interrogation signal. Moreover, the split electrode approach offers a possibility to significantly reduce the impact of the small working electrode on impedance measurement. The increase in driving voltage is therefore an acceptable concession for EIS based sensors. The validity of the approach is not affected by the choice of measurement topology, though the impact of parasitic couplings remains less problematic for SMU-based measurement.

A consequent extension of the four-electrode setup using split electrodes at both terminals in combination with SMU-based measurement is simultaneous measurement at both terminals. In the presence of parasitic losses from the electrolyte, the double measurement provides further information, since the current at both terminals is different. However, the additional effort required to facilitate the double measurement is not feasible on large electrode arrays, and a measurement at a large, common counter electrode may not provide information relevant to the purpose of the array. Better candidates for double sided measurement would be Coulter counters and interdigitated electrode structures, where the two terminals operate more symmetrically. Accounting for stray currents then allows for detection of violations of symmetry, such as cells

positioned off centre. In that case it may even be beneficial to introduce intentional current sinks for stray current to maximise the information gained from the double measurement.

For large, high density arrays it is not feasible to use intentional current sinks, since the cumulative effect would produce an unacceptably large parasitic load. Instead, it is necessary to avoid unintentional current sinks to minimize the impact of parasitic couplings on measurement. At the same time, however, it is important to shield the active electrodes from noise sources. This presents a trade-off, since the structures used to shield the electrodes function by serving as sink for the noise. As such a sink also poses a pathway for the interrogation signal, parasitic coupling to the shielding is inevitable. Since the impact of parasitic coupling depends on the measurement topology, the electromagnetic barrier also needs to be considered in light of the measurement topology.

The most common approaches to implement shielding are to connect the conductors to ground or to leave them floating. Floating conductors are only efficient as barrier against interference with a wavelength shorter than the conductors. EIS sensors operate in a frequency range with wavelengths that are longer by several orders of magnitude, so floating conductors would serve as an intermediate point for electromagnetic coupling. Although the isolating layers on both sides of the shielding help to increase the distance the coupling has to reach, the exposed surface area is increased, possibly increasing the total coupling. By attaching the conductors to a fixed potential such as ground, the interference is diverted, providing a reliable barrier, but also contributing a considerable sink for the interrogation signal.

The shielding needs to be present for every electrode and the related circuitry, so the exposed area is large for an electrode array. This means that the impact is reduced for SMU-based measurement, since the influence of parasitic couplings declines with the distance from the active electrode. The effect can, however, also be partially compensated in a conventional measurement topology by partitioning the barrier and connecting only the parts relevant to active electrodes to ground. If such a division is implemented, the conventional measurement topology has an advantage over the SMU-based measurement approach, since in the latter case there is a potential difference between the working electrode at signal level and the shielding at ground potential. To avoid this, an SMU-based topology needs to connect the conductor barrier to a copy of the interrogation signal as well. Since the interrogation signal is already distributed as input to the measurement TIA, it is also readily available for the purpose of shielding. However, this presents a considerable additional load for the distribution network which the driving circuits need to support. It is also important to ensure the driver for the distribution network functions as sink for interference. Partition of the barrier into smaller units may help to achieve the required performance by limiting the active parts of the shielding and hence effective interference. More importantly, however, the partitioning reduces the impact of the barrier on the sensing field in the electrolyte. Because there is coupling between the shielding and the

Figure 8.20: Current flow for uniform potential in working electrode plane: a) without obstacle; b) with obstacle

electrolyte, the applied signal distorts the current in the electrolyte and consequently the measuring field.

If an isolating layer exists between the conductors of the barrier and the electrolyte, the coupling may be small enough to tolerate the change of the sensing field. In some cases, the deviation may even be beneficial and improve the focus of the sensing to an area of interest. The focusing effect is illustrated in Figure 8.20 using the extreme case where the entire plane of the working electrode is at the same potential and the counter-electrode stretches infinitely parallel to the plane. If the volume between the plane of the working electrode and the plane of the counter-electrode is homogenous, the current flows in a straight line from one plane to the other, as indicated in Figure 8.20a. In contrast, if the current can only flow into a small working electrode, it spreads with the distance from the electrode. If an obstacle, such as a cell, is present in the electrolyte, it is detectable because it forces the current around it and thus changes the impedance. The more the current is spread, the smaller the fraction of it which the obstacle can affect. Since the surrounding current prevents the current spread as illustrated in Figure 8.20b, the current is more effectively blocked by the obstacle, increasing the measured impedance. Hence, the focussed current has the potential to provide a better sensitivity.

The cost for this improvement is that a higher total current is required, since the entire volume has to be filled with current. It is therefore not feasible to apply the signal to the entire array at the same time. However, it is not necessary to achieve a focusing effect throughout the electrolyte to improve measurement results, as the relevant sensing area is limited. For an electrode array used in a biosensor, the important obstacles to detect are close to the surface, as the cells settle on the sensor. In particular, a major limiting factor for the detection of cells is the bounded seal resistance to current bypassing a cell that has settled on the electrode. Consequently, it is sufficient to counter this bypass current to achieve an improvement. The focus of the sensing current then only needs to reach up to the height of the gap between the electrode and the cell to accomplish this.

In case of an SMU-based EIS measurement system, the change of the electric field due to active shielding conductors may already cause a minor focusing effect. However, if the structure is designed purely for shielding, the coupling to the electrolyte is too weak to meaningfully improve the sensitivity of the sensor. To achieve good coupling to the electrolyte, an additional electrode carrying the focusing signal is required. It is not possible to reuse the sensing electrode if a split electrode setup is used, since the bounding electrode needs to carry a defined, separate potential, so in this case a third electrode is required. The additional electrode needs to surround the working electrode to ensure a focusing effect from all sides, though the electrode can be formed by separate elements if the gaps between them are sufficiently small. Independent of the design of the focusing electrode, the effect achieved using the interrogation signal directly is limited. Because a gap at the edge of the sensing electrode is inevitable, current spread cannot be entirely avoided. To counter the current spread further, it is necessary to increase the amplitude of the focusing signal. This creates a potential gradient and thus current in direction of the sensing electrode, effectively forming a pinch effect for the measured sensing current.

For simulation of the effect, a single ring electrode was chosen around a circular working electrode to make use of the rotational geometry. Figure 8.21 shows simulation results for a setup using a working electrode with a radius of $14\text{ }\mu\text{m}$. The focusing ring electrode has an inner radius of $18\text{ }\mu\text{m}$ and an outer radius of $22\text{ }\mu\text{m}$. A passive electrode with an inner radius of $26\text{ }\mu\text{m}$ is used to represent inactive electrodes in the array. The setup was investigated for frequencies from 10 Hz up to 100 MHz and the results varied considerably due to the frequency dependent properties of the electrodes. The results shown are for a frequency of 100 kHz and a focusing potential twice as high as the interrogation signal. In Figure 8.21a, the electrolyte is homogenous and no obstacles are present. The focusing effect is most apparent in the dashed lines representing the current flow in the electrolyte. Though the spread is not entirely avoided, it is significantly bound by the flow from the focusing ring electrode. The limiting factor is the smaller surface area of the ring electrode compared to the working electrode, leading to inferior

Figure 8.21: Simulation results for setup with current pinch electrode: a) without cell model; b) with cell model

coupling to the electrolyte. Nonetheless, the equipotential surfaces, illustrated through solid lines in Figure 8.21, are clearly altered near the electrode surface. In particular, above the focusing ring the equipotential surface becomes nearly horizontal, signifying near vertical current.

To investigate the impact on the sensitivity with respect to cell detection, the simulations were repeated with an obstacle. The cell was represented by a hemisphere with a radius of $14\text{ }\mu\text{m}$ centred $2\text{ }\mu\text{m}$ above the working electrode. The hemisphere model was chosen to represent a cell that has largely settled on the electrode, while the distance from the electrode represented an imperfect seal. The interior of the cell was assumed to be a perfect isolator in order to keep the focus on the pinch effect and allow clearer interpretation of the frequency response. As the results in Figure 8.21b show, a considerable current is able to bypass the cell, though the focusing ring is capable of forcing the current into a near vertical flow with a narrow spread near the cell up to a height of about $8\text{ }\mu\text{m}$.

The pinch effect is also visible in the effective impedance of the setup, shown in Figure 8.22. Below 10 kHz, the impedance is dominated by the electrode/electrolyte impedance of the working electrode, and neither the obstacle nor the pinch electrode have a significant impact on the measurement. The influence of the obstacle on the observed absolute impedance becomes relevant at around 20 kHz, whereas the pinch effect only develops a significant impact around 70 kHz. With respect to the absolute impedance alone, the focusing electrode does not improve the sensitivity of the sensor, though the magnitude of the impedance is increased. In particular, for a ring potential twice as high as the interrogation signal amplitude V_I , the impact of the obstacle is reduced beyond 200 kHz. The reason for this behaviour is that once the coupling of the focusing electrode is strong enough, current is flowing close to the surface from the ring electrode into the working electrode. However, for a pinch potential of $2V_I$ the obstacle leads to a considerable phase shift in the observed impedance over a wide frequency range from 10 kHz to almost 10 MHz. Consequently, the sensitivity is increased even though the difference in absolute impedance is reduced. For a pinch potential that is equal to V_I on the other hand, the phase sensitivity to the obstacle is largely unchanged, whereas the sensitivity of the absolute impedance is slightly increased for frequencies larger than 100 kHz. The highest total improvements in sensitivity are therefore expected for pinch potentials between V_I and $2V_I$, although the exact value depends on the exact structure of the setup and the properties of the obstacle.

Even if the thin ring electrode used in the simulation provides a significant change in current flow, a larger surface area is still desirable to improve the coupling to the electrolyte. However, even the electrode used in simulation requires significant space that may not be available in an electrode array. The area also presents a problem because cells may be settling on the focusing electrode instead of the actual sensing electrode. A solution to these challenges is to re-use

Figure 8.22: Bode plot of effective impedance with current pinch electrode

neighbouring electrodes instead of adding a dedicated electrode. Due to the large available electrode surface area, this allows a considerable pinch effect using the interrogation signal without amplification. The drawback to this option is added complexity to the electrode circuits, which may lead to an increase in the noise level. Nonetheless, using the existing electrodes is preferable unless a method is found to reliably draw the cells onto the sensing electrode.

For the implementation of the focusing effect, a scaled interrogation signal is needed. Because the interrogation signal itself is available at the electrode in an SMU-based measurement system, this can be generated locally with minimal effort. Alternatively, the higher amplitude signal can be used for distribution, and the lower amplitude interrogation signal generated locally. This scaling of the signal may then be integrated with the TIA. The pinch effect can also be achieved for a conventional measurement topology. An inverted copy of the signal is needed in this case for the focusing electrode to achieve the inward flow. Consequently, a distribution network has to be added to the design, leading to both topologies requiring approximately the same effort.

Though the improvements on the electrode structures and circuits were first considered before the development of the biosensor presented in Section 8.1, they were omitted from the design. This choice was made to avoid the unnecessary complexity in the proof of concept chip. Though the additional effort necessary for the integration of the improvements to the electrode structures would have been relatively low since many of the circuits are already present in the design, the required labour still had to be considered. Since the additional novelty was beyond the scope of the design, this added need for labour was prohibitive. The electrode shielding, for instance, was not implemented to limit the time required for layout.

Nonetheless, the enhancements to the electrode structures show considerable promise, particularly in combination with the SMU-based EIS approach. The improvements are capable of reducing the influence of the electrode/electrolyte interface significantly, avoiding the related nonlinear behaviour and variability which are detrimental for biosensors using EIS. The approach is not without risks, as strong voltage peaks over the interface may result, driving destructive processes. If these damages are avoided, the measurement results are dominated by the impedance between the connecting electrodes. Since this impedance contains most information for EIS biosensors, this dominance helps to raise the desired signal against the background. In combination with the current pinch approach, the measurement can moreover be focussed on a targeted zone above the electrode, further increasing the signal from cells settling on the electrode.

The presented enhancements are not unique to SMU-based EIS measurement, as they can be implemented with conventional measurement topologies as well. However, the infrastructure provided by the SMU architecture is a natural match for the improved electrode structures and reduces the required effort for their implementation. Hence, it appears worthwhile to pursue the techniques together in the future.

8.7. Summary and Conclusion

The studies on the first and second generation biosensors indicated that measurement topology may have a considerable impact on measurement quality. The dominant topology in commercial systems uses a driving counter electrode and measures at the working electrode. The advantage of this configuration is separation of concerns, as issues of driver design do not interfere with the design of the measurement circuits. However, measurements with this topology do not account for current lost to other channels than the working electrode. An SMU-based setup sacrifices accuracy and precision, but measures the entire driving current. Moreover, the better current concentration at the working electrode means that measurement is more localized. The effect of distant structures is minimized, reducing the impact of parasitic coupling. As a beneficial side effect for high frequency designs, the concentration of measurement avoids propagation delays on long lines. If sufficiently fast mixer circuits are

realized, EIS interrogation at GHz frequencies is possible without the need to consider signal propagation. Based on the SMU topology, a proof of concept biosensor was developed. The focus of the design was on the distribution network and the enhanced driving capability that the previous biosensor was missing. Though space for improvements remains on many levels, the design met its specification in simulations.

The change in measurement topology has consequences for layout and electrode design. Due to time constraints, these could not be incorporated in the developed biosensor. Instead of passive ground shielding, the active signal needs to be shielded with an identical signal to prevent capacitive losses. If the signal is surrounded by an amplified version of itself, however, it presents a way to improve the focus of the measurement on the area above the electrode. Although similar approaches are possible with conventional EIS systems, the SMU-based setup provides the necessary infrastructure with minimal additional effort. The SMU topology is also extendable to three and four electrode measurement setups. This allows to reduce the influence of the working electrode and the counter electrode on the measured impedance, though the space between the working and the sensing electrode leads to a scaling factor in the results. Since a relative change in impedance between the electrodes is the value of interest, this has considerable potential to improve measurement results.

SMU-based EIS sensors are a promising approach for microelectrode arrays. The change of topology has the potential to minimize the impact of parasitic couplings on measurement results by keeping the active currents concentrated at the sensing site. As an additional benefit, the more localized structure shows a path to avoid critical propagation delays, allowing very high interrogation frequencies. SMU-based measurement can also be gainfully combined with enhanced electrode structures to raise the relevant information above the baseline measurements. Though challenges remain, the new design implementing the SMU topology presents a valuable step in realizing these advantages.

Chapter 9: Conclusions and Future Work

The development of EIS biosensors presents a wide range of challenges. In this work, some of these challenges were identified and addressed, although shortcomings remain and need to be confronted in future work. Many directions of research also remain to be investigated for future improvements of measurement speed, quality or depth of results.

In spite of their unique advantages, it has not been conclusively shown yet if microelectrode arrays are the best approach to CTC detection and analysis. Of the devices discussed in this Thesis, CTC detection was so far demonstrated only with the first-generation biosensors. The minimal integration of these devices allows for flexible use of the sensor, but the lack of signal buffers leads to relatively poor signal integrity, and parasitic couplings add up over long signal paths. The second-generation biosensor improves significantly in these areas due to integrated amplifier chains, and the overall design is considerably cleaner. The design of the amplifier chains reduces the flexibility of the sensor, though, and the output signal was not normalized, hampering the interpretation of results. The third-generation biosensor had a similar degree of integration as the second-generation chip, and had comparable advantages and disadvantages. However, because it was designed for use with different measurement topologies, it was more flexible in this respect. In particular, the architecture supported SMU-based EIS measurement, which improves on the locality of the measurement and thus reduces the impact of parasitic couplings far from the measurement site. In the longer term, the goal is not just the development of amplifier chains for EIS measurement, but rather the creation of an integrated measurement system for cell analysis. For this purpose, each individual building block needs to be selected from the possible solutions and implemented. Above all, however, the weaknesses of the current sensors have to be acknowledged so that they can be corrected before moving on to more complex integration.

Although the effect is less pronounced than originally anticipated, parasitic couplings still affect impedance measurement dramatically. The topic of parasitic couplings had previously been neglected, and more work is needed to provide a conclusive framework for evaluation. The impact from parasitic coupling increases with the size of microelectrode arrays and the bandwidth of the interrogation signal, but the effects do not have to pose a major issue if they are considered in conception and design. However, significant problems may arise if the influence of parasitic coupling and current leakage is ignored.

Even in the absence of parasitic coupling, the detection capability of current sensors is limited by small signal strength and a lack of uniformity of the electrode characteristics. The lack of signal strength is due to cells not covering the electrodes well, allowing most of the current to bypass and hence not changing the impedance much. This may be partially fixed by advancements in surface modification, as the cells can be drawn to adapt better to the shape of

the electrodes. The work on aptamer SAMs for cell capture is already providing a significant step in this direction, and the technique also improves selectivity of the sensor. The lack of electrode uniformity is harder to solve, as it is largely due to post-processing issues, and improvement of the processing parameters is not straightforward. A contributor to this problem that may be easier to solve is the influence of parasitic couplings on electrode behaviour. The influence of the coupling varies with the electrode, and this variation can be minimized by reducing the total impact of parasitic coupling.

Further possible ways to improve EIS measurement and microelectrode array design could not be included in this Thesis, as they would have led to excessive side-tracking. Digital stimulus signals based on pseudorandom sequences offer considerable increases in measurement speed because of their high bandwidth in spite of a moderate implementation effort. The use of such digital signals may even open up an alternative route to high speed ADCs for signal evaluation, as the frequency response can instead be analyzed based on channel estimation techniques known from communication technology. The main advantage of this approach is that the viable bandwidth may be increased drastically. Whereas a moderate increase of EIS bandwidth is unlikely to provide additional information due to cell transparency in the respective frequency region, frequencies in the GHz range may be able to elicit responses from dielectric relaxation mechanisms.

With respect to manufacturing and packaging, active CMOS microelectrode arrays may benefit from a shift to autocatalytic electrode growth instead of lithographic methods of structuring. Although electroless plating is mostly used on non-biocompatible metals such as metal or chrome, thin gold layers are also widely deposited in this manner. The necessary process parameters may be challenging to find, and sources are scarce on the quality of electrodes fabricated in this manner, but the technology has the potential to produce uniform, self-sealed electrodes with perfect alignment to the CMOS technology. Independent of the method of growing the electrode structures, the results from surface modification with aptamers have been very promising and deserve to be expanded on. In particular, the combination of different doubly selective surface layers to achieve a repulsive behaviour on the oxide and an attractive behaviour on the electrodes may achieve a push-pull effect on cells, greatly increasing the chances of cells settling in the desired place.

A major concern for development is that the system should move towards real-life applications in order to have a positive impact on health care. However, the path to practical applications is still long, as the reliability of sensors is insufficient for clinical use. Independent of the cells targeted by the application, the electronics and interface characteristics of the microelectrode array must first be optimized to ensure that cell attachment can be differentiated from electrode to electrode variations in measurement results. Surface functionalization may not be necessary, but the prevention of cell adhesion in undesirable locations is needed in many

applications to avoid cell loss and unclear signals due to the cells settling only partially on the electrode. Once the dimensions and the interface of the electronics are settled, the packaging must be developed fully. Especially the microfluidics must be optimized to minimize the loss of sample in the leads to the measurement chamber. With respect to the application of CTC detection and characterization, the preparation of the sample itself also requires further research. Once the technical challenges are met, extensive biological studies on cell cultures are needed to prove reliable detection of CTCs. Further studies are required using blood samples spiked with a known quantity of CTCs to show the enumeration capability of the system. Only after the reliability is demonstrated on these model samples, clinical studies are tenable.

Until the technical challenges are overcome, cell studies can serve to guide development, and they are vital in the study the impact of surface functionalization on cell attachment. However, cell experiments provide additional information only related to cell behaviour and impact on measurement. While this information is crucial for biosensors, it is of minor importance for the design of the integrated measurement circuits, as the required specifications of the circuits are already given through the behaviour of the electrodes and the effective spreading resistance. The impact of a cell on these characteristics is either small, or the major deviations can be taken as indicator for the presence of the cell. Consequently, only few experiments with cells were conducted as part of this Thesis, as the focus was on the integrated circuit design. Nevertheless, it is worthy to reiterate that studies with cell samples will eventually be required to validate the circuits.

In the short term, it is more important to integrate as many components as possible of the measurement chain, as described in Section 5.3, since external measurement is undesirable due to higher noise levels and component costs. Up to this point, merely the electrode setup and the electrode selection can be considered settled, as even the TIA may require optimization. However, both the second-generation biosensor and the new design are suitable for expansion, although the new design requires additional tests to prove its viability. The first step of any expansion is a finalization of the TIA chain in order to fix the frequency response between signal source and signal conversion into a digital form. Once the frequency response is fixed, the control scheme needs to be fixed to allow for independent development of the managed components and avoid unnecessary redevelopment of test board software. Ideally, a standard serial link is implemented to ease communication with a microcontroller on the test board.

If parallel development is not possible due to a lack of manpower, the next module to be implemented is the signal source. This task comprises two overlapping steps, however, as in a first step, a decision on the interrogation signal needs to be made before the hardware implementation can be completed. For cell detection and analysis, a relatively narrow frequency band between 10 kHz and 1 MHz ought to be sufficient, as the critical frequencies have been found around 100 kHz. At these frequencies, measurement is fast enough for a basic sensor to

operate with single frequency measurement. Nonetheless, for flexibility and to minimize the amount of difficult analog design, a DAC based synthesis appears as the best option for a physical realization of the signal source. The largest amount of effort is necessary to design a suitable ADC. A bandwidth of at least 10 MHz is required for clean sampling, although it may be possible to work with lower sample rates if advanced techniques such as purposeful undersampling are used. The final signal evaluation is purely digital, and hence development and testing are fairly straightforward. Nonetheless, a separate test chip for signal source, ADC and signal evaluation may be advisable to avoid overloading the sensor with untested equipment.

In all development, it must be considered that simple components with slightly less performance may be preferable to high performing modules that are not ready for mass production, as the primary goal has to be a working system, rather than an ideal system. Biosensors have great promise because of their possible impact on health care, and the sooner the technology becomes ready for market, the more patients can benefit from it.

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