

Institut of Bio- and Nanosystems (IBN)
Bioelectronics (IBN-2)

***Design and development of
amplifier electronics for
silicon-nanowire biosensors***

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Chapter 1

Introduction

Bioelectronic sensors represent a major field of interest in current research. Their ability to detect and transduce biological processes like DNA-hybridization or electrical cell signals into measurable currents generates numerous applications. For example, they can be used for non-invasive monitoring of electrical cell signals in cell cultures or for the detection of very low concentrations of biological molecules. Several approaches to improve the recording properties of bioelectronic sensors have been conducted in recent works. The sensors were optimized for higher sensitivities [1], for a low noise behavior [2] and for high spatial recognition by using large sensor arrays [3]. A common feature of those sensor systems is that they work in environments, which inherently generate large amounts of noise. At the same time, the sensed signals are very weak and must be separated from disturbing effects.

Signal processing offers a wide range of methods to enhance data computation. Especially since the break through of digital signal processing (DSP) methods numerous fields, e.g. telecommunications showed great benefit from this development. By using DSP the signal detection and computation can be significantly improved. It allows to filter out relevant information, to compensate measurement errors, or provides a statistical description of signals. These are properties which are especially interesting for sensor applications, where the information is often masked by large noise components. The measurement system frequently incorporates additional errors, which must be separated from the signal of interest, and statistical descriptions can be used to estimate noise processes or to detect weak signals.

This makes it interesting to use signal theory approaches in conjunction with biological measurements. Especially in the detection of action potentials of electrically active cells the potential of signal theory usage was recognized. By using wavelets [4, 5] and nonlinear energy operators [6], weak action potentials of neuronal cells can be detected. Also in the domain of cell characterization and molecule detection signal theory approaches can be found. A basic approach for biological measurements is to observe the resistivity changes of sensors itself [7]. Other methods gather additional information about the measurement system by quantifying its complex impedance [8, 9]. The impedance is affected by several elements of the measured system. By developing models, these parameters can be extracted to deduce further information about the

bioelectronic system. This was already done for the detection of a protein layer thickness [10] or to explain cell transfer functions [11].

The objective of this thesis is to develop a measurement system which can be used for current and future sensor devices in bioelectronic applications. The measurement system should be able to record resistive changes and to measure the complex system impedance. Furthermore, the measurement results need to be explained and interpreted by the development of system specific models.

This thesis is structured in six chapters. After this introduction, the different types of sensors and measurement approaches are introduced in chapter two. In the third chapter the signal processing performed by the developed amplifier system is described theoretically. The fourth chapter deals with the used setup and the measurement processes. The fifth chapter presents results of the development of the amplifier system and some applications. Furthermore, the measured systems are described by signal theory to allow a deeper understanding. Finally, a conclusion and outlook for future improvements and measurement approaches is presented in chapter six.

Chapter 2

Fundamentals

2.1 Sensor devices

In the past 30 years the development of transistors which can couple ionic charges to electrical currents showed great progress [12]. For biological systems, they allow non-invasive monitoring of cells or they can be used to detect numerous molecules. However, there is a new trend which can be observed in this domain. Measurements on wire based sensors showed higher sensitivities and thus promise to enhance molecule detection. In the following sections both types of sensors will be introduced. Their principal functioning will be explained and their differences will be pointed out.

2.1.1 The field-effect transistor

A field-effect transistor (FET) is a device which basically converts a voltage into a current and at the same time offers an infinite input resistance. In figure 2.1(a) a principal layout of a FET is shown. It mainly consists of a semiconducting channel which is connected to the outside by two highly doped regions. These are the source and drain terminals of the FET. The gate consists of a highly conducting material which is separated from the channel by a very thin insulation layer, mostly made of SiO_2 .

The resistivity of the channel can be altered by a voltage applied to the gate terminal. Since the insulation between the gate and the channel is very thin, a voltage on the gate can accumulate or deplete charged carriers in the channel region. The number of free carriers in turn determines the conductivity of the channel. Thus if a constant voltage is applied between the drain and source terminals, the channel current (mostly called drain current I_D) can be altered. There is often an additional terminal, which is connected to the substrate of the FET to ensure the isolation of the drain and source region (see [13] for further informations on FETs).

These devices are extensively used in all sorts of electronics. In order to utilize them for biological measurements the gate metalization is omitted. Instead the biological sample is directly

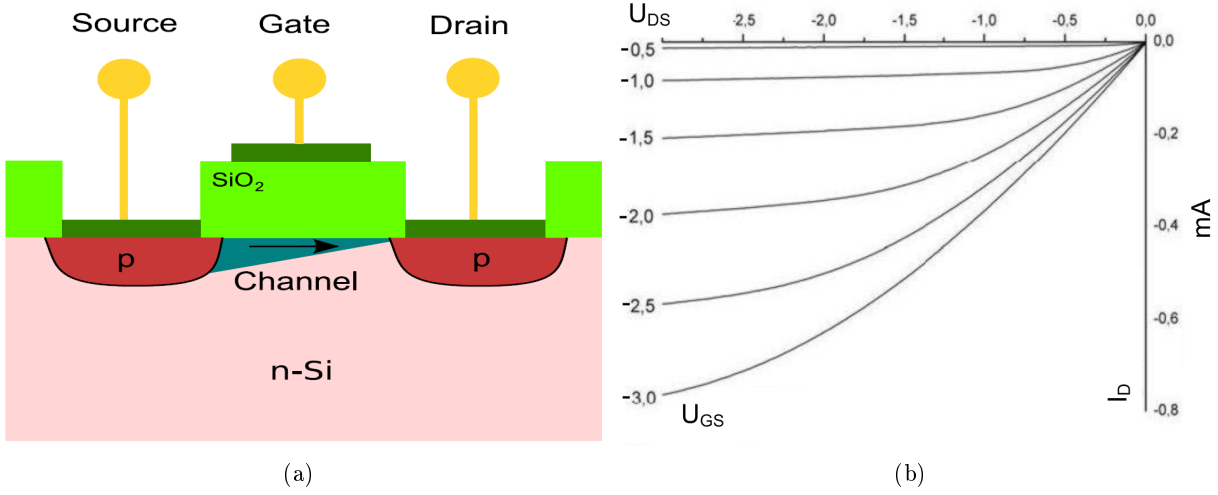


Figure 2.1: Schematic drawing (a) and output characteristic (b) of a p-FET. (source: [14])

brought in contact with the insulating oxide [15]. The charge of ions contained in the biological sample creates a surface potential which has the same effect as applying a gate voltage. For example, if there is a high concentration of positively charged ions on the gate surface of a n-FET, negative charges will accumulate in the channel due to electrostatic attraction. Thus the charges on the gate can control the number of free carriers in the channel. Therefore these kind of FETs are called ion-sensitive field effect transistors (ISFET). This property makes them very interesting for the coupling to biological systems. In these systems most signals or changes are expressed by alternating ion concentrations. The type of measurements which can be performed with such a device will be discussed in section 2.2.

To perform a measurement with the ISFET devices, they are immersed into an electrolyte. By the use of a reference electrode, the electrolyte has a defined potential. An Ag/AgCl electrode is used, since it provides a constant potential in the electrolyte solution. This approach allows to apply a gate voltage on the ISFET devices. Thus they can be characterized like any other FET. In figure 2.1(b) the so called output characteristic is shown. It shows the drain current I_D as a function of the drain-source voltage U_{DS} . By changing the gate-source voltage U_{GS} the current I_D can be controlled. This is the typical behavior of every FET.

An important property of a FET is the so called transconductance g_m . It is defined by:

$$g_m = \frac{\partial I_D}{\partial U_{GS}} \quad \text{low signal definition:} \quad g_m = \frac{i_D}{u_{GS}} \quad (2.1)$$

This value is important since it gives a relation of the gate voltage to the drain current of the FET. To assess its value the so called transfer characteristic of a FET is recorded. In figure 2.2(a) such a characteristic is shown for an ISFET device used in this thesis. It shows a set of current traces for variable U_{GS} and fixed U_{DS} . By differentiating the plot (equation

2.1) the transconductance shown in figure 2.2(b) can be obtained. It has a maximum around $-1.5 \leq U_{GS} \leq -1.0$ V. Within this range the input signal is amplified the most.

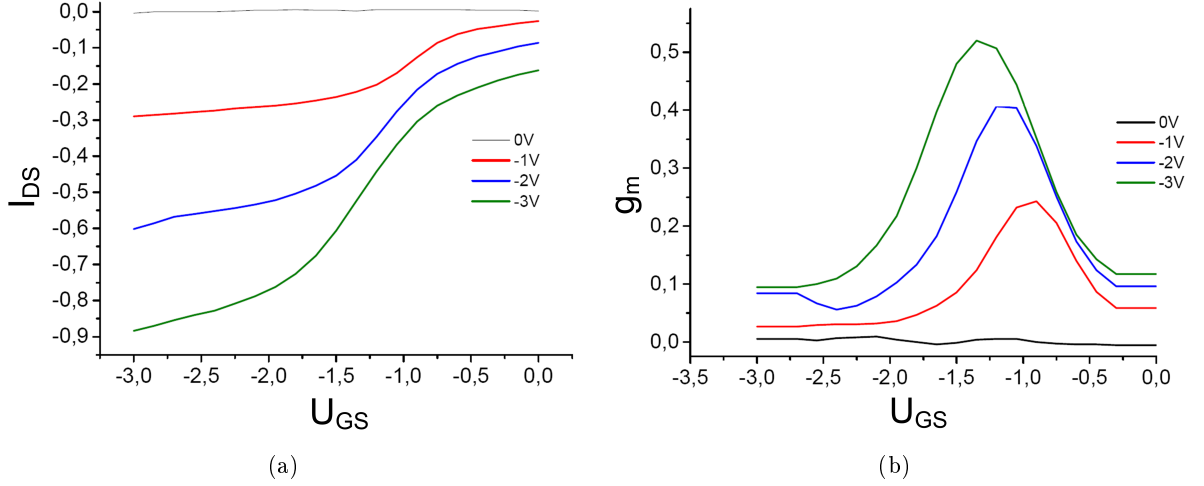


Figure 2.2: **(a)** Transfer characteristic and **(b)** transconductance of an ISFET. (source: [16])

To use the maximum transconductance of the FET, it is biased to a so called operating point. An operating point consists of a pair of gate-source and drain-source voltages. In comparison to these bias voltages, the actual input signal must be small so that the FET always remains in the region of the operating point. This allows to linearize the FET behavior at this point and simplifies its mathematical description. For example in the case of the ISFET in figure 2.2(b) the operating point is chosen to be $U_{GS0} = -1.5$ V and $U_{DS0} = -3$ V because there the transconductance has its maximum. For typical measurements the input voltage is around 20 mV. Thus the FET will always operate in the region around U_{GS0} and U_{DS0} of the characteristic. This part is approximated by a linearization and can be described by low level values. By convention these are written in small letters. Thus g_m can be simplified by just calculating the ratio of the drain current and the gate voltage as was already shown in equation 2.1. A very detailed introduction on the use of low level signals to describe FETs can be found in [13]. It will be used in section 5.6 to model the sensor behavior. The importance of the operating point will be further discussed in section 5.5.

2.1.2 Wire-based sensors

Wire-based sensors represent an alternative to ISFET devices. They use the same physical effects as FETs do, but they have a different geometrical structure. A simple approach to produce them is based on silicon on insulator (SOI) wafers. The structure of such a wafer is shown in figure 2.3(a). It consists of a three layers stack: the normal silicon substrate, the buried oxide and the top silicon. To build a sensor, the top silicon is etched in such a way that only the small wires

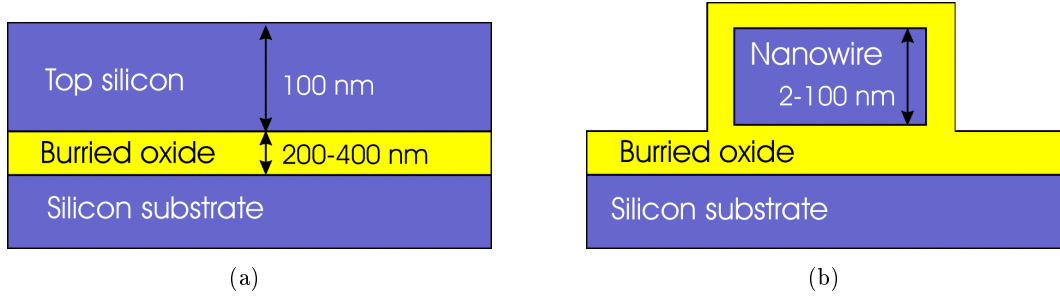


Figure 2.3: Silicon on insulator wafer before (a) and after (b) processing to nanowire devices.

remain. By using SOI-wafers the wires are already isolated to the substrate and only a thin SiO_2 layer has to be grown on top to form the front gate. The result is shown in figure 2.3(b). In reference [17] nanowires based on this approach were used.

Several papers have been published [1, 18, 19, 7, 20] demonstrating the performance of these wires in bio-sensing applications. Since they are very promising, the amplifier system in this thesis was designed for these devices. However, only microwires could be used for the measurements, since they are easier to produce. They mark an intermediate step to nanowires, but their behavior should be similar enough to be used as a first test system.

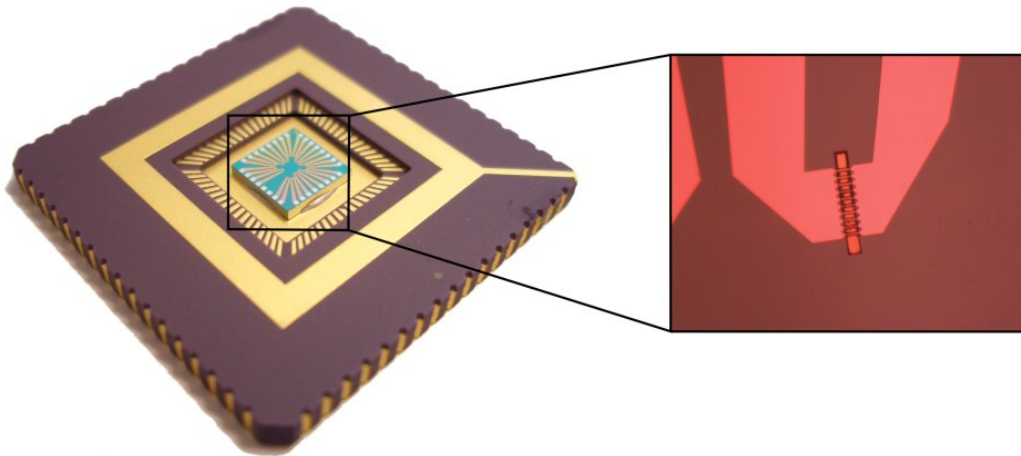


Figure 2.4: Example of a microwire chip, which is not completely encapsulated. The inset shows a set of ten $5 \times 2 \mu\text{m}$ microwires.

Figure 2.4 shows a not fully encapsulated microwire chip. In the inset ten $5 \times 2 \mu\text{m}$ microwires in parallel can be distinguished. The entire chip is covered by a passivation of several 100 nm to ensure a good insulation to the electrolyte. Only inside the rectangle around the microwires the oxide is limited to 2 nm. This is the front gate which consists of the electrolyte - wire interface.

There is a second gate which is between the substrate and the wire and is called the back gate. It can be used to set the operating point as it was described in subsection 2.1.1.

To set the operating point, the microwires characteristics are required. In figure 2.5(a) the output characteristic of a microwire is shown. It is very similar to the one for a p-FET device (figure 2.1(b)). Thus it is reasonable to regard the wires as FETs. Their transconductance is shown in figure 2.5(b). Obviously it is by one decade smaller than for the ISFET devices (figure 2.2(b)) and thus the size of the current is also smaller. Furthermore the operating point cannot be chosen freely. To measure the characteristics very high voltages were applied to the chip. This is no problem, since the voltages are separated by the 400 nm BOX. In the case of a real measurement, however, the natural oxide of the front gate is only around 2 nm thick and cannot isolate such high voltages. Therefore U_{DS} should always be below two volts. For most measurements an operating point of $U_{BG} = 20\text{ V}$ and $U_{DS} = 1.3\text{ V}$ was chosen.

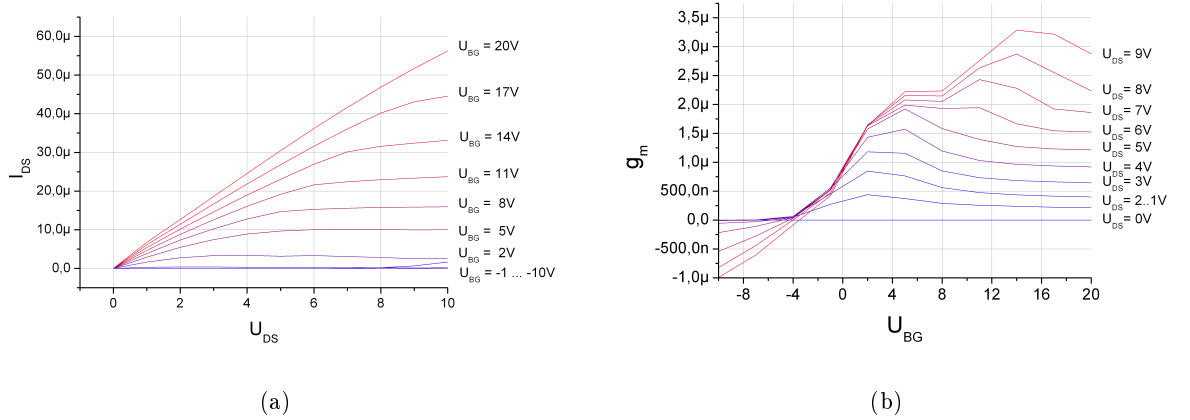


Figure 2.5: (a) Output characteristic and (b) transconductance of a $5 \times 2\text{ }\mu\text{m}$ silicon wire.

Another important difference to ISFETs is the high resistance of the microwire devices, which lie in the $M\Omega$ range. This imposes additional requirements on the amplifier system, which must have a significantly higher input impedance to measure correctly.

2.2 Measurement methods

The devices presented in the previous sections are very versatile sensors. They can be used for numerous detection and monitoring applications. However, there are two general approaches how the sensor signals can be interpreted. The so called potentiometric and impedimetric sensing. These two approaches will be presented in the following subsections and some measurement examples will be given.

2.2.1 Potentiometric sensing

The probably best known and thus often applied approach is to measure the resistive change of the sensor. Since the sensors behave like FETs, a change in resistance must be due to a different amount of charge on the gate. Thus the change in resistivity is directly linked to the gate potential, why this approach is called potentiometric sensing.

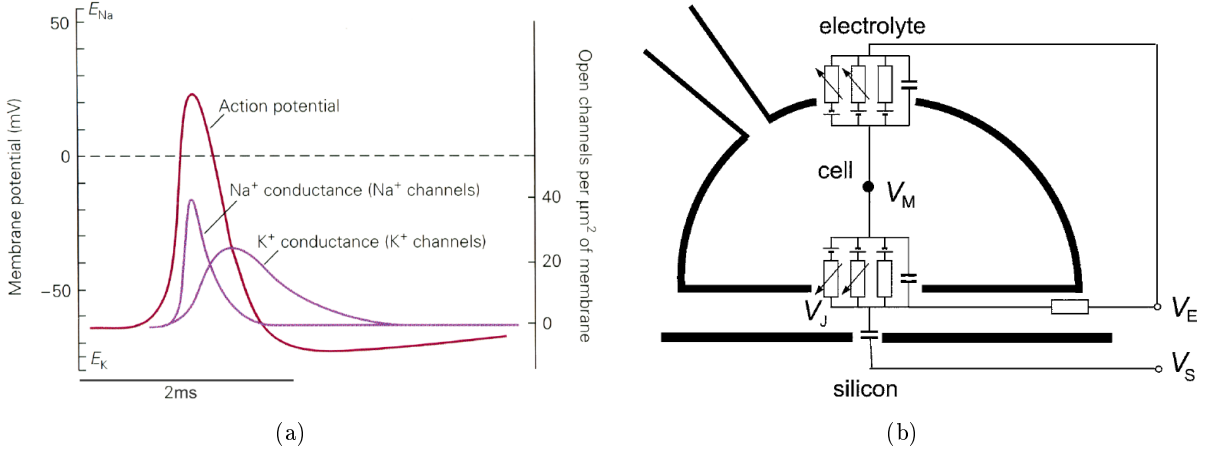


Figure 2.6: (a) Action potential of a neuron by the sequential opening of Na^+ and K^+ channels. (b) The point-contact model describing changes in the cell potential. (source: [2, 21])

Depending on the measured system a change of the gate charge can have several reasons. In cell measurements, action potentials can be measured by this approach. An action potential is a traveling wave of rapid changes in the membrane potential. Electrogenic cells use this for signal propagation. To generate an action potential a cell abruptly changes the ion flow through its membrane. Therefore it uses so called ion channels which gate a specific ion type to the inside or outside of the cell. This changes the ion composition and concentration on both sides of the cell membrane and thus alters the cell potential V_M . The cell potential in turn determines when the ion channels will open. In figure 2.6(a) an example of an action potential is shown. Three different phases can be distinguished. During the depolarisation the sodium concentration inside the cell increases which leads to a rapid elevation of the cell potential. When it reaches a critical value, the K^+ channels become permeable and gate the potassium outward the cell. During this repolarisation phase the membrane potential drops below its initial value. Finally the cell enters the hyperpolarisation phase where it restores the ion gradients. To electrically describe this process the so called point-contact model (see [21]) was developed, which is shown in figure 2.6(b). Between the cell and the transistor surface, there is a cleft of 60-100 nm [22] filled with electrolyte solution. In the model this cleft is described by the seal conductance G_J . The membrane itself is characterized by Hodgkin-Huxley elements which are modeling the overall membrane characteristics and the properties of every specific ion channel i . The ionic current through specific protein channels is determined by the equilibrium potential E_i^{JM} which

is shown by batteries and the conductivity G_i^{JM} , modeled by changeable resistances (see [2] for a more detailed description). To calculate the junction potential $V_J(t)$ the following (simplified) equation can be used:

$$V_J(t) = \frac{1}{G_J} \left(C^{JM} \cdot \frac{d(V_M(t))}{dt} + \sum_i G_i^{JM} (V_M(t) - E_i^0) \right) \quad (2.2)$$

This potential is also the gate voltage which can be measured. Obviously a higher seal resistance will lead to a higher junction potential which in turn is easier to detect. The seal resistance mainly depends on the quality of the cell adhesion. Experiments with action potentials already have been discussed in several publications in detail [2, 3].

In the case of chemical detection, the charge must be coupled to the target molecule itself. For example for DNA-detection, a buffer with a pH between seven and eight is used. In this pH region the DNA carries an overall negative charge. In order to bind the DNA to the chip surface, there are complementary, single stranded pieces of DNA immobilized on the gate. By adding the matching single stranded DNA to the electrolyte solution, the DNA can hybridize at a temperature of 37 °C and increase the negative charge on the gate surface. However, due to the so-called counter-ion screening effect the effective charge on the gate is even lower [23]. Small counter ions in the electrolyte solution are attracted and cause macroscopically uncharged layers of DNA. Therefore only charges inside the debye length can be detected. This problem can be reduced by performing the measurement in low ionic electrolytes. However, this decreases the probability of hybridization due to the electrostatic repulsion between the DNA strands. Another problem is that the DNA is bound to the surface by linker molecules. They increase the distance of the charged DNA to surface and thus also lower the signal. Nevertheless small changes in the sensor current can be measured and have been used for the detection of very low DNA concentrations [1]. An important advantage of this approach is that it requires no labeling of the DNA. This simplifies the readout and increases the measurement speed [24]. Furthermore this approach is usable for the detection of numerous molecules like penicillin [25], anti-bodies [26], proteins [10] or as a pH sensor [27]. The exact measurement process for DNA hybridizations has been described on several occasions [28, 29]. In reference [30] a model is presented based on the potential differences at the sensor surface to describe the sensor sensitivity.

2.2.2 Impedimetric sensing

The other measurement method is to record the overall, complex impedance of the system. Therefore it is called impedimetric sensing. The complex impedance of the system results from the impedance of all elements which are located between the electrode and the gate of the sensor. To describe this impedance so-called transfer functions are very suitable. They are defined by the ratio of the output and input voltage of a system. Thus, each point of the transfer function magnitude describes the system attenuation at a given frequency. They can be easily measured and calculated which makes them ideal for comparisons between theory and experiments. In contrast the potentiometric approach measures the changes of the charges on the gate surface.

The different measurement methods will be further described in chapter 3.

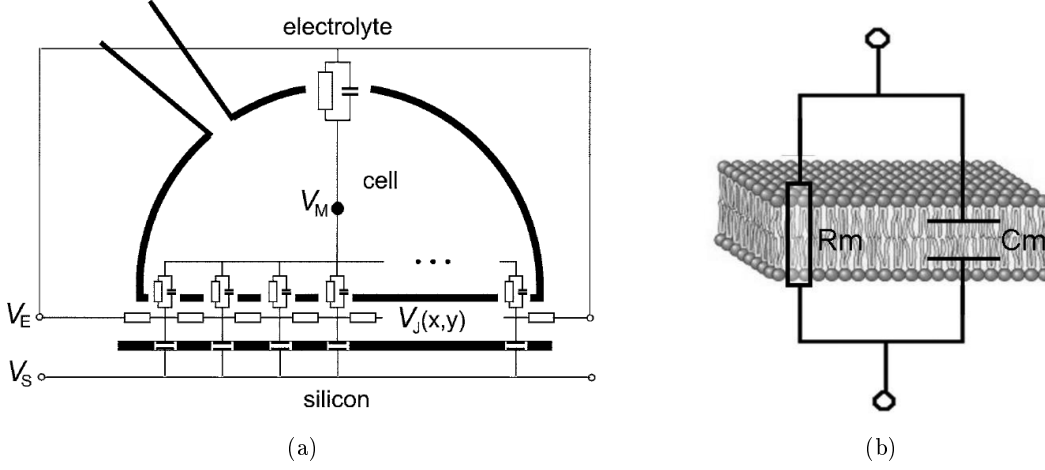


Figure 2.7: (a) Area contact model which describes the complex impedance of an adhered cell. (b) An electrical description of the cell membrane. (source: [21, 16])

Impedimetric sensing can be used in biological measurements to deduce informations of the cell characteristic. In a first approximation a cell membrane can be described electrically by a resistor and a capacitor in parallel (see figure 2.7(b)). Since these elements are very dependent on the surface size of the cell they are most often expressed as area-related values. Thus a typical specific capacitance of a membrane is $c_M = 1\mu Fcm^{-2}$ and the specific conductivity of an artificial lipid bilayer is around $g_M = 10^{-8}Scm^{-2}$. In a natural membrane with embedded proteins the specific conductivity can vary from $g_M = 1Scm^{-2}$ to $g_M = 10^{-4}Scm^{-2}$ [31]. This concept to describe the cell membrane is also used in the area contact model. It describes the impedance of the entire cell [21] as is shown in figure 2.7(a). Since the cell is described by infinitesimal elements, the model takes local variations into account. Beside the membrane, the seal resistance and the gate capacitance are taken into account. In section 5.8 a model based on a similar approach will be used to deduce the size of the elements out of the transfer function. This will permit to collect more information about the cell than just potentiometric measurements would provide.

Impedimetric sensing can also be used for DNA detection. The advantage in comparison to the potentiometric method is that it eliminates side effects like pH, temperature drift or electrolyte composition [9]. As in the potentiometric measurement the gate surface is covered by single stranded pieces of DNA. However, the transfer function is measured before and after the DNA hybridization. The measurements show a change in the transfer functions. This change allows to distinguish between a perfect match or a single mismatch of the added DNA to the single strands on the gate surface [9]. The change in the impedance is due to a geometrical capacitance effect [23], which appears in addition to the change of the surface charge on the gate. The additional DNA molecules displace the electrolytes which leads to a higher "effective" insulator thickness. This decreases the "effective" gate capacity, which can be detected by the

complex impedance. Therefore the layer on the gate surface is modeled as a set of a capacitor and a resistor in parallel [10]. This method is called ex-situ, since the transfer functions before and after the hybridization are compared. In contrast potentiometric measurements allow an in-situ monitoring, since the resistive change is recorded during the DNA hybridization.

Chapter 3

Signal processing theory

The primary goal of this thesis was to develop an amplifier which can be used in conjunction with wire-based sensors. In a first section the general concept of the amplifier is presented. The requirements, assets and drawbacks of this concept are discussed. Then the data computation is explained by using signal theory. To measure the transfer functions of the sensor, a Lock-In and a noise based approach are introduced. Furthermore there is a short introduction on how the noise power density of a signal can be estimated.

3.1 The amplifier concept

Previous amplifiers for ISFET devices in our institute are designed as purely analog systems. For the amplifier developed in this thesis another approach was chosen. The basic measurement principle is shown in figure 3.1. Since all sensors in this thesis acted as a transistor they are symbolized as FETs. The sensor system was excited by a voltage source, which applied its signal to the electrolyte solution via an Ag/AgCl electrode. The electrolyte solution could contain different kinds of biological samples (e.g. cultured cells or biomolecules). The output signal of the sensor was fed into a first amplification stage. There a current-voltage conversion and a compensation step were done. The second amplification stage had a high, adjustable gain which adapted the signal to the input range of the analog to digital converter (ADC). Finally, the signal processing was done in digital form.

The goal of this amplifier concept was to convert the measured signal as soon as possible into a digital form. Thus the most part of the signal processing was performed digitally. There are several reasons to prefer a digital rather than an analog signal processing:

- Digital signal processing (DSP) is much more flexible than analog signal processing. Especially in an experimental work it is favorable to be able to change the signal path at any time. An analog circuit remains fixed and can only be changed under big effort.

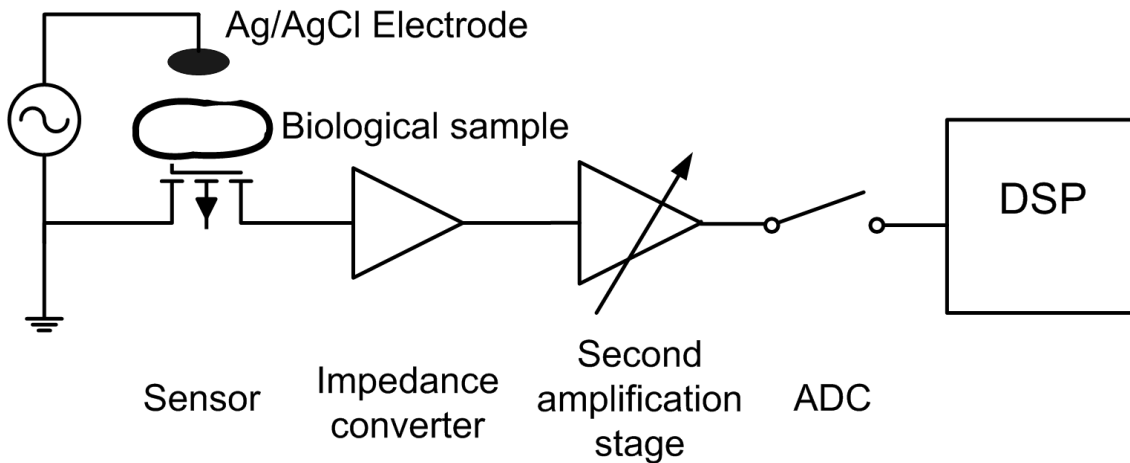


Figure 3.1: Principle of a bioelectronic measurement with the new amplifier.

- There are less components which can interfere with the signal. For example in analog systems, multipliers can add frequency dependent offsets to their output signal. As it is discussed in section 3.2 this adds a systematic error to the measurements. In a digital multiplier such an error does not exist. Another obvious example is that in digital systems there is no drift.
- Digital systems perform calculations at a very high precision. In contrast to this, the precision of analog systems is limited to a ratio of about 60 dB [32]. At higher signal ratios they do not work reliably any more.
- In digital systems it is very easy to retain and save signals. Once a signal is digitalized it can be used, processed and reviewed as often as desired. This makes it possible to use the same sampled signal to extract several, different informations (for example magnitude, phase, constant component, noise ...).
- The design of analog filters for low frequencies is often associated with numerous problems. The devices are bulky or do not exist in the required sizes. In DSP systems appropriate filters can be generated for every frequency range.
- In analog systems device saturation or non-linear behavior must be considered. Due to the calculation precision and the perfect linearity (e.g. of a multiplication) this is not an issue in digital systems.

However, a drawback of DSP is the bandwidth of digital systems. ADCs are very limited in their maximum sampling rate and even if a high sampling rate can be attained, the data processing is limited by the high computational load. The only way to increase the sampling rate is to diminish the resolution. This is the reason why analog systems are dominant in high frequency applications. For biological measurements sampling rates between 10 kHz and 1 MHz

are sufficient. These can be easily attained by currently available ADC components.

Besides these considerations there were some more specific requirements which the amplifier should fulfill. They were due to the new devices and to the experience gathered by previous measurement systems in our group.

- The ISFETs used in our group have a g_m of about 0.1 mS. In contrast several publications [33, 34, 35] on nanowires report a g_m in the range of 1 - 0.1 μS . Thus the current of the sensors drops by two to three decades which required a higher amplification.
- The new amplifier should be very flexible in terms of being easily adaptable to different measurement conditions. It should give an insight in the signal path for problem elimination and be extendable to support new measurement approaches.
- The measurement system should be as transparent as possible. All electronic influences and disturbances should be shielded such that only the information of interest appears in the measurements.
- It should not have a limitation concerning low frequencies. Thus measurements below 0.1 Hz should be no problem.

With the concept shown above most of these requirements could be fulfilled. Thus the following sections discuss the detailed signal computation. Thereafter the realization of the digital and analog parts is presented.

3.2 The Lock-In amplifier

The key feature of a Lock-In amplifier is the high noise rejection. To achieve this, only a small part of the signal spectrum is analyzed. The device under test (DUT) is stimulated by a sinusoidal signal at a constant frequency. Provided that the system is purely linear, only a sine of the same frequency but with different amplitude and phase is seen at the output. Unfortunately, the sensor devices used in this work add an inherent noise signal which is often bigger than the signal of interest. Hence the information of the sensor is buried under noise. To be able to still detect a signal, the Lock-In uses very narrow band filters which only pass the frequency of interest. All remaining parts of the spectrum are discarded. In the case of white noise for example, this can greatly diminish the noise power without decreasing the signal power. Therefore it can be said that a Lock-In increases the signal-to-noise ratio (SNR) of a signal.

In the following subsections the basic Lock-In amplifier is described. It is explained how the filtering is realized and how the polar values of a transfer function are deduced. Furthermore there is a subsection on how to design filters which meet the desired specifications for this application.

3.2.1 Signal computation

In principle a Lock-In amplifier is a very narrow bandpass filter. To measure a transfer function the center frequency of this bandpass is shifted accordingly. In figure 3.2 a schematic shows the signal processing in a Lock-In.

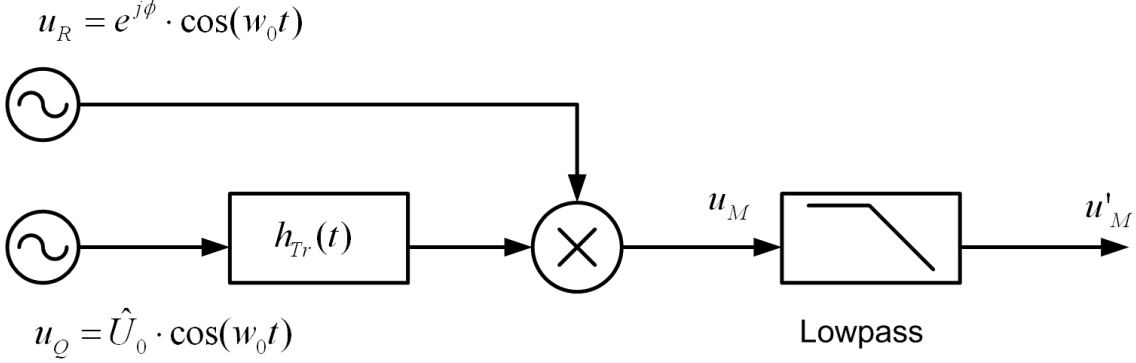


Figure 3.2: Basic Lock-In amplifier.

u_Q is the source signal, which stimulates the sensor. The sensor itself is described by the transfer function h_{Tr} . Its output signal is multiplied with a reference signal u_R . Finally after a low pass filtering step, u'_M is the output signal of the Lock-In. As source signal a perfect sine wave is used. To achieve a good performance of the Lock-In, the quality of the sine wave is crucial. This will be shown later, when the system is tested with different signal sources (see subsection 5.1.3). The source signal is defined by:

$$u_Q = \hat{U}_0 \cdot \cos(w_0 t) \quad (3.1)$$

To select the frequency which the Lock-In should filter out, a reference signal is used. It is defined by:

$$u_R = e^{-j\phi} \cdot \cos(w_0 t) \quad (3.2)$$

The significance of the phase shift, denoted by ϕ will become apparent later. As a result of the Lock-In signal processing only the real part of u'_M is of interest since the imaginary part cannot be measured. In the following an equation for $Re\{u'_M\}$ is derived. The goal is to find a relation to deduce the complex quantity $h_{Tr}(t)$ from $Re\{u'_M\}$. To calculate it, the source signal u_Q is convolved with the transfer function $h_{Tr}(t)$ and multiplied by the reference signal u_R . To simplify the calculation u_M is transformed in the frequency domain. As a convention all quantities in the time/frequency domain are written in lower/upper case:

$$u_M = [u_Q * h_{Tr}(t)] \cdot u_R \quad \longleftrightarrow \quad U_M = [U_Q \cdot H_{Tr}(jw)] * U_R \quad (3.3)$$

3.2. THE LOCK-IN AMPLIFIER

U_Q and U_R are represented by two dirac impulses in the frequency domain. U_R has the additional factor $e^{-j\phi}$ due to the phase shift.

$$u_Q = \hat{U}_0 \cdot \cos(w_0 t) \quad \circ \text{---} \bullet \quad U_Q = \frac{\hat{U}_0}{2} [\delta(w - w_0) + \delta(w + w_0)] \quad (3.4)$$

$$u_R = e^{-j\phi} \cdot \cos(w_0 t) \quad \circ \text{---} \bullet \quad U_R = \frac{e^{-j\phi}}{2} [\delta(w - w_0) + \delta(w + w_0)] \quad (3.5)$$

By using the filter property of the dirac impulse in U_Q equation 3.3 is written as:

$$U_M = [U_Q \cdot H_{Tr}(jw_0)] * U_R \quad (3.6)$$

Since $H_{Tr}(jw_0)$ is a constant, it is factored out:

$$U_M = [U_Q \cdot H_{Tr}(jw_0)] * U_R = H_{Tr}(jw_0) \cdot U_Q * U_R \quad (3.7)$$

Thus only the part of the transfer function with the desired frequency is determined. This is easy to understand, since the linear system $H_{Tr}(jw)$ is only excited by one frequency w_0 .

The convolution of U_Q and U_R results in a shift in the frequency spectrum. Since the reference and the source signal oscillate at the same frequency, new signal components appear in U_M at $w = 0$ and at $w = \pm 2w_0$:

$$U_M = H_{Tr}(jw_0) \cdot \hat{U}_0 \cdot \frac{e^{-j\phi}}{4} [\delta(w + 2w_0) + 2\delta(w) + \delta(w - 2w_0)] \quad (3.8)$$

By substitution of the transfer function by its complex coefficients $H_{Tr}(jw_0) = a_{w_0} + jb_{w_0}$ the equation is extended to:

$$U_M = \frac{\hat{U}_0}{4} e^{-j\phi} (a_{w_0} + jb_{w_0}) [\delta(w + 2w_0) + 2\delta(w) + \delta(w - 2w_0)] \quad (3.9)$$

In figure 3.2 it can be seen that the signal U_M is low pass filtered. Assuming that a perfect low pass filter is used and that all components are attenuated, except $w = 0$, only the DC part remains:

$$U'_M = \frac{1}{2} \hat{U}_0 e^{-j\phi} (a_{w_0} + jb_{w_0}) \delta(w) \quad \bullet \text{---} \circ \quad u'_M(t) = \frac{1}{2} \hat{U}_0 e^{-j\phi} (a_{w_0} + jb_{w_0}) \quad (3.10)$$

As mentioned above, only the real part can be measured. By eulers identity and the extraction of the real values, $Re\{u'_M(t)\}$ is written as:

$$Re\{u'_M(t)\} = \frac{1}{2} \hat{U}_0 [a_{w_0} \cos\phi + b_{w_0} \sin\phi] \quad (3.11)$$

$Re\{u'_M(t)\}$ contains both, the real and imaginary parts of the transfer function. By adjusting the phase of the reference signal the complex parts are separated at the Lock-In output. Thus two multipliers are used in order to compute the complex transfer function. They are fed by two sine waves one of which was shifted by 90° . Based on these two outputs the magnitude and

phase are calculated. This representation of the transfer function is very convenient and is used for all transfer function plots in this work. Thus finally, $h_{TR}(t)$ is calculated by:

$$h_{Tr}(t) = \frac{2}{\hat{U}_0} \cdot \left(\text{Re}\{u'_M(t)\}|_{\phi=0^\circ} + j\text{Re}\{u'_M(t)\}|_{\phi=90^\circ} \right) \quad (3.12)$$

Under ideal circumstances, $h_{TR}(t)$ can be exactly determined by the Lock-In. By using DSP several error sources can be eliminated which otherwise would appear in an analog signal processing. However, since the measured system is always analog some error sources cannot be avoided which are further discussed in section 5.4. Furthermore one error source remains on the digital side which is due to the filtering. In the calculation an ideal filter, which blocks every frequency except the DC component was assumed. Unfortunately, also in digital systems such filters can only be approximated. Therefore in the next section the design of appropriated filters is discussed. A detailed theoretical description of pass band filters (this is what the Lock-In mainly consists of) can be found in [36].

3.2.2 Filter synthesis

The noise rejection of a Lock-In is determined by its low pass filtering capabilities. The stronger the attenuation, the better the noise components can be suppressed. In the ideal case only the DC component of a signal should pass the filter system.

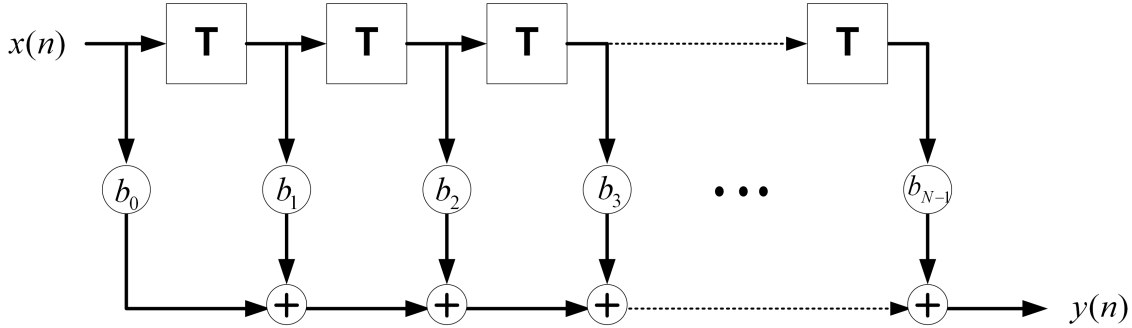


Figure 3.3: General schematic of a FIR filter.

There are mainly two types of filters in digital signal processing: infinite impulse response (IIR) and finite impulse response (FIR) filters. Figure 3.3 shows the general schematic of a FIR filter. Its output corresponds to the weighted sum of a time-delayed input signal $x(n)$. The input is delayed several times by a time constant T . In the following T will always be the inverse of the sampling rate: $T = \frac{1}{f_{\text{Sample}}}$. Those retained values are multiplied by constant factors of b_x ($x \in [0; N-1]$) and summed up. N is the number of filter coefficients. To determine the impulse response of such a system, it is stimulated by a dirac impulse $\delta(n)$:

$$\delta(n) = \begin{cases} 1 & n = 0 \\ 0 & n \neq 0 \end{cases} \quad (3.13)$$

The response to such an input would be:

$$y(0) = b_0 \quad y(1) = b_1 \quad y(2) = b_2 \quad \cdots \quad y(N-1) = b_{N-1} \quad (3.14)$$

It is apparent that the impulse response of a FIR filter is always limited to the number of filter coefficients. Deduced from this the impulse response can be written generally as:

$$h_{FIR}(n) = b_0 + b_1 \cdot z^{-1} + b_2 \cdot z^{-2} + \dots + b_{N-1} \cdot z^{-(N-1)} \quad (3.15)$$

IIR filters are mainly realized the same way. Except they use an additional, weighted feedback signal. This feedback can lead to a not disappearing signal at the output of the filter, even if there is no input any more. So in contrast to FIR filters their stability is not guaranteed under all circumstances. To simplify the filter design and to avoid considering stability issues, only FIR filters were used in this work.

To use a FIR filter, appropriate coefficients b_x have to be chosen which determine the transfer function of the filter. However the degree of freedom is limited. As will be shown later there is a trade off between a high attenuation and minimizing the number of filter coefficients. The process of finding the optimum coefficients is called filter synthesis. There are several ways to deduce appropriate coefficients from a set of filter requirements. A very straightforward approach is to first define the optimum transfer function. Recalling equation 3.9, only the constant component at $f = 0$ should pass the filter. Thus the perfect transfer function would be a simple dirac impulse: $H(jw) = \delta(w)$. An example of such a transfer function is shown for discrete frequencies in figure 3.4(a). There the transfer function is plotted versus the normalized frequency. The normalized frequency is defined by $f_{normalized} = \frac{2 \cdot f}{f_{sample}} = 2 \cdot f \cdot T$. It is used, because the frequency behavior of any FIR filter is determined by the sampling rate. As it was shown in figure 3.3, T is the delay between the filter coefficients. Depending on the size of T , the transfer curve can be stretched or squeezed over the frequency. Therefore it is more convenient, to use the normalized frequencies rather than giving absolute values.

In order to deduce the filter coefficients the transfer function is transformed to the time domain. The result is the impulse response of this ideal filter. By comparing it with equation 3.15 the coefficients can be deduced.

A dirac impulse $\delta(w)$ is represented in the time domain by a constant value $h(n) = 1$ (see figure 3.4(b)). To achieve a perfect filter like in figure 3.4(a), an infinite number of coefficients would be necessary. Even if the number of coefficients would be very big ($N \gg 0$) the spectrum of the filter would still more resemble to a Si-function ($Si(x) = \frac{\sin(x)}{x}$). So this ideal filter can only be approximated. To avoid an amplification by the filter system all FIR filters must be normalized so that they fulfill the following constraint:

$$\sum_{i=0}^{N-1} b_i = 1 \quad (3.16)$$

In order to meet equation 3.16, the coefficients are divided by N . Thus the filter described above corresponds mathematically to calculating the mean value of the signal why it is called the mean filter. To evaluate the performance of this approach, it is compared to a so called brick wall filter.

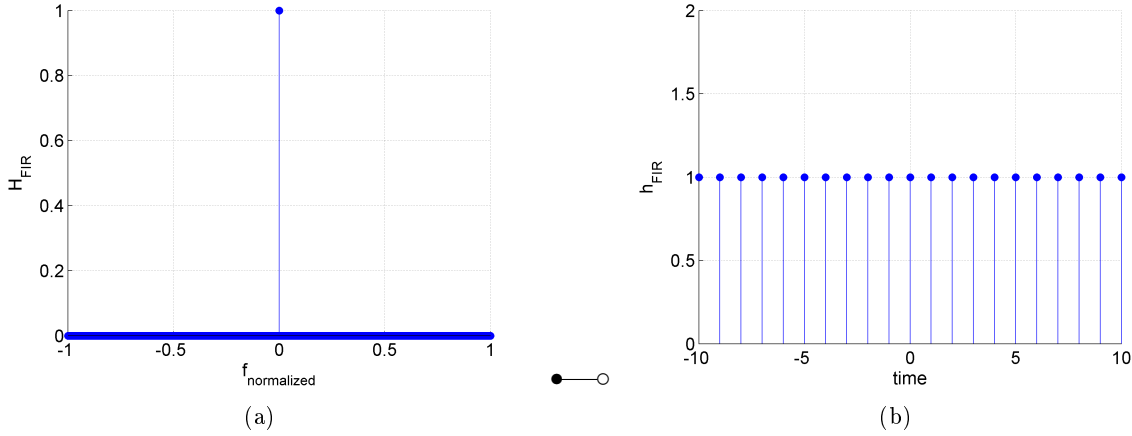


Figure 3.4: Transfer function and impulse response of a perfect mean FIR filter. **(a)** In the frequency domain, only the part at $f = 0$ can pass the filter. **(b)** The corresponding impulse response is a constant value.

The brick wall filter describes an ideal way of low pass filtering. Because of its wide use, it is described in great detail in literature [36, 13, 37]. The transfer function of such a filter is shown in figure 3.5(a). It is an ideal filter in the sense that it has a perfect pass band and a perfect attenuation in the stop band. The impulse response and the filter coefficients correspond to a Si-function (see figure 3.5(b)). The smaller the pass band in the frequency domain is, the longer the Si-function takes to decay. In this case, the pass-band should be very small, so the Si-function is widely spread.

Figure 3.6 shows the transfer functions of the mean and the brick wall filter for 2000 coefficients. Obviously the brick wall filter has a better attenuation of approximately 20 dB at higher frequencies. This is surprising since the transfer function of the mean filter is just the special case of the brick wall filter with zero bandwidth. Thus one could expect that the overall attenuation is similar. The reason is probably due to the approximation of the filters. Ideally an infinite number of coefficients would be needed to exactly produce the spectra in figure 3.4(a) and 3.5(a). However since always only a limited number of samples can be computed, the number of filter coefficients is also limited. The transfer characteristic of a mean filter with only a few

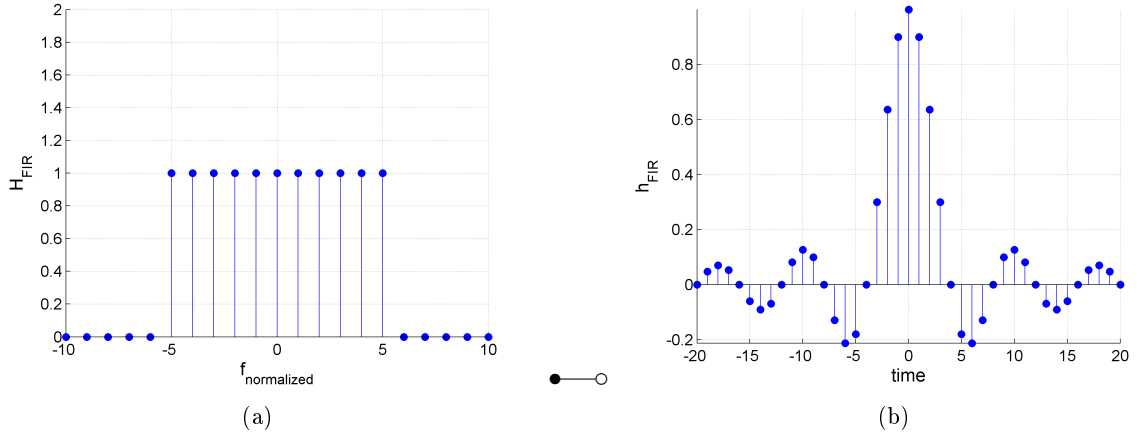


Figure 3.5: **(a)** The transfer function of an ideal low pass filter is a rect-function. **(b)** The corresponding impulse response is a Si-function.

coefficients will be more like a Si-function than a dirac impulse. In contrast the transfer function of the brick wall filter realization will not differ so much from its ideal shape. In the time domain the Si-function of the brick wall filter decays to zero. In contrast the mean filter always stays constant. Thus if the coefficients are cut at a specific point to keep the system usable, then there will always be a bigger error to the ideal case, for the mean filter.

In a Lock-In a strong attenuation is important since it determines how much the output noise can be decreased. Therefore the brick wall filter is used in the Lock-In development of this thesis. It is always favorable to minimize the number of coefficients, since this minimizes the computational effort and the amount of samples.

This comparison of the mean and the brick wall filter was done to give a feeling for the filter design in a Lock-In and to show which parameters are of interest for improvements. The filters are probably the most important parts, since they strongly affect the Lock-In results and since they offer several optimization possibilities. It turns out, that even though the design approach seems to be very straightforward, the resulting transfer function can be quite different from the expected one. As another improvement possibility it should be noted that all these filters are of first order. To improve the filtering, higher orders are of interest. By processing the signal several times, the filter order is increased. This improves the overall attenuation and the filter slope. However as it will be shown later, the computational effort is then also increased.

3.3 Maximum length sequences

A very simple and straightforward way to determine the transfer function of a system would be to measure the impulse response. By an additional fourier transformation the transfer function

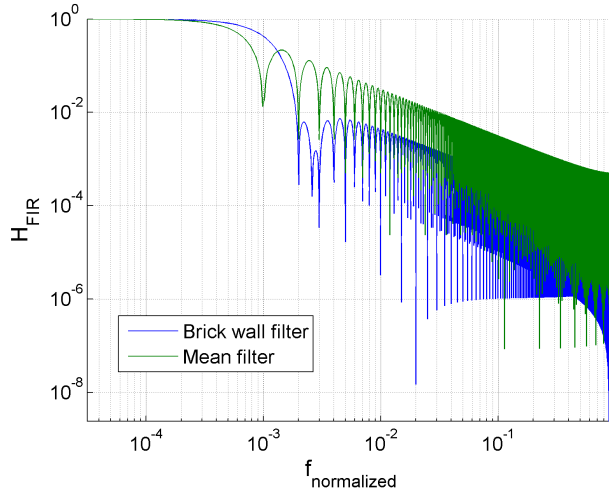


Figure 3.6: Comparison of a mean and a brick wall filter, both with 2000 coefficients.

could be calculated. However, to obtain a good impulse response the quality of the stimulating dirac pulse is crucial. It should have an energy which is high enough to have a good separation of the impulse response from noise. On the other hand it should be as short as possible, which limits the energy and by this the noise separation. Also the pulse height is generally limited, since most systems only stay linear in a limited range. Therefore, this kind of measurement is not widely used, but these problems can be circumvented by using maximum length sequences (MLS).

3.3.1 MLS properties and generation

To retrieve the impulse response, a MLS (also called M-sequence) is applied to the input of a measured system. An example of a MLS is shown in figure 3.7(b). The signal has only two output values (+1 and -1). Every pulse (positive and negative) is a multiple of T_p in length, and the whole sequence is periodic with T . M-sequences have several interesting properties. They have a perfect flat spectral power density function. Thus, in terms of spectra, they behave like white noise. This also implies that they contain all frequency components equally. This is important to correctly measure transfer functions, since all frequencies must be stimulated.

To generate a M-sequence, shift registers can be used as shown in figure 3.7(a). The input is calculated by a modulus 2 operation of the weighted outputs of the n shift registers. It is apparent, that the registers can have $L = 2^n - 1$ different states. Thus a MLS of order n is of length L . However, not every combination of coefficients gives a correct MLS. Actually there exists only a very limited number of M-sequences which fulfill all properties described above. Correct start values for the shift registers can be found in [38]. There are further methods for MLS generation by combination of shorter M-sequences described by [39].

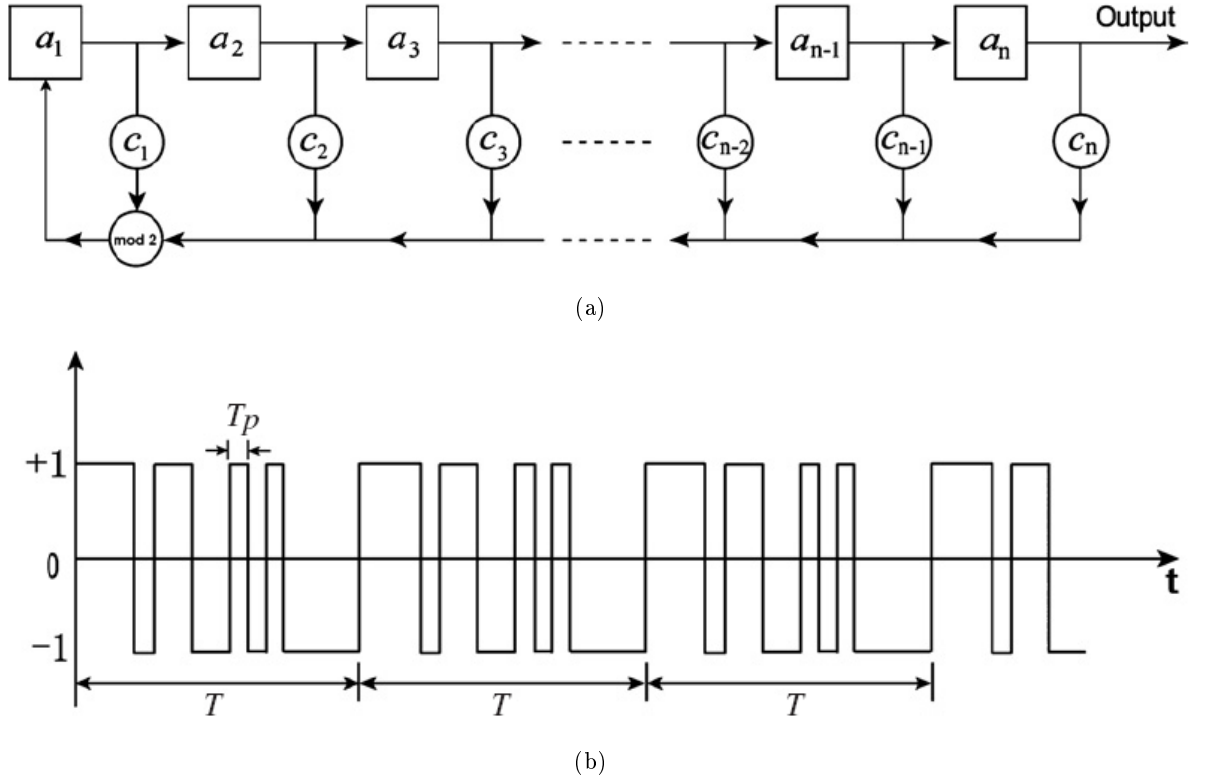


Figure 3.7: **(a)** A MLS can be generated by a series of shift registers. The weighted sum of the registers is taken modulo two and is used as input for the system. **(b)** Example of a MLS. The pulses are multiples of T_p long and the whole signal is periodic with T . [8]

The autocorrelation function of a M-sequence has some important properties. An example for a 10th order MLS is shown in figure 3.8. Except for a peak in the middle at $t = 0$, the autocorrelation function is nearly zero. This shows that the signal is uncorrelated as it would be the case for white noise. As defined in [36] the autocorrelation function $\varphi_{ss}(\tau)$ of a signal $s(\tau)$ in the time domain is given by:

$$\varphi_{ss}(\tau) = s^*(-\tau) * s(\tau) \quad (3.17)$$

Since M-sequences are always real, $s^*(-\tau)$ can be replaced by $s(-\tau)$. To describe the normalized autocorrelation function of a M-sequence $m(k)$ analytically, it can be approximated by:

$$\varphi_{mm}(k) = \frac{1}{L} \cdot m(-k) * m(k) = \frac{L+1}{L} \cdot \delta(k) - \frac{1}{L} \quad (3.18)$$

A comparison with figure 3.8 shows that equation 3.18 is in good agreement with an exact solution (see [40, 41] for further details). As it will be shown, this special shape of the autocorre-

lation function plays a key role in the calculation of the impulse response. Another property of a MLS is that it contains exactly one -1 more than +1's. This is also important for the calculation (see appendix A).

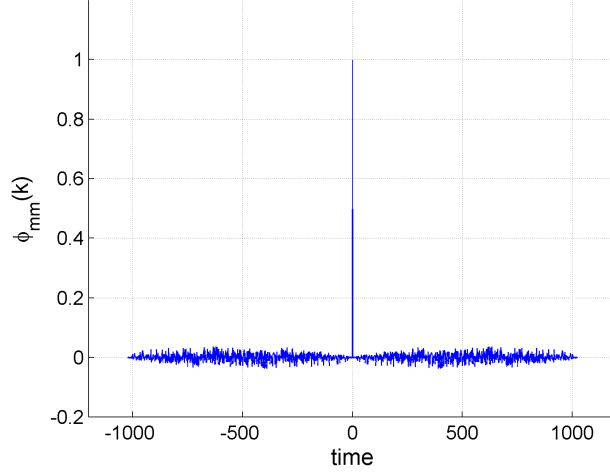


Figure 3.8: Autocorrelation function of a MLS with $n = 10$.

3.3.2 Autocorrelation function and impulse response

In the following an equation which allows to calculate the impulse response of a system is developed. A M-sequence $m(k)$ will be used as input. This results in

$$y(k) = m(k) * h(k) \quad (3.19)$$

$y(k)$ as the measured response of the system. By a convolution with $m(-k)$ it becomes:

$$h'(k) = y(k) * m(-k) = [m(k) * h(k)] * m(-k) \quad (3.20)$$

Then equation 3.18 can be substituted in $h'(k)$:

$$h'(k) = L \cdot h(k) * \varphi_{mm}(k) = h(k) * [(L + 1) \cdot \delta(k) - 1] = (L + 1) \cdot h(k) + (-h(k)) * 1 \quad (3.21)$$

In equation 3.22 the definition of a discrete convolution is shown.

$$g(k) = s(k) * n(k) = \sum_{m=-\infty}^{\infty} s(m)n(k-m) \quad (3.22)$$

If $n(k)$ is set to one in equation 3.22 and is substituted it in equation 3.21 it becomes:

$$h'(k) = (L + 1) \cdot h(k) - \sum_{m=-\infty}^{\infty} h(m) \quad (3.23)$$

Furthermore, since $m(k)$ is a M-sequence, the negative sum of $h(k)$ can be replaced by the sum of $y(k)$ (the prove for this relation can be found in the appendix A) :

$$h'(k) = (L + 1) \cdot h(k) + \sum_{m=-\infty}^{\infty} y(m) \quad (3.24)$$

The variable of interest is $h(k)$. So the equation is rearranged and $h'(k)$ is substituted by it's definition in equation 3.20. Furthermore, the borders of the sum are changed, since $y(k)$ is only non-zero for $1 \leq k \leq L$:

$$h(k) = \frac{1}{L + 1} \cdot \left(y(k) * m(-k) - \sum_{m=1}^L y(m) \right) \quad (3.25)$$

Finally by replacing the calculation of the convolution by the definition in equation 3.22 and by adjusting the borders of the sum $h(k)$ becomes:

$$h(k) = \frac{1}{L + 1} \cdot \left(\sum_{n=1}^L y(n)m(n - k) - \sum_{m=1}^L y(m) \right) \quad (3.26)$$

By this equation the impulse response of a system can be calculated (see also [40]). The only values needed are the output $y(k)$ of the system and the M-sequence $m(k)$ applied to its input. The result $h(k)$ is the impulse response of the measured system. Although no dirac impulse is applied at the input, the impulse response is finally obtained. The central idea is to use the resemblance of the autocorrelation function to a perfect dirac impulse, to calculate the impulse response. As a simple mathematical description this can be written as

$$m(k) * h(k) * m(-k) \quad \hat{=} \quad \delta(k) * h(k) \quad (3.27)$$

The benefit of this approach is that the problems described at the beginning of this section have been circumvented. By distributing the energy over the length of the MLS, the system will always stay linear. Furthermore, there is no need of an accurate pulse generator. The M-sequence can simply be generated by a DAC.

In equation 3.26 the first term calculates the autocorrelation function and thus the impulse response of the system. The second term is a correction factor. As it can be seen by the calculations above it results from the remaining constant component of the autocorrelation function. It should be noted that the description of the autocorrelation function (in equation 3.18) is only an approximation. Thus the calculated impulse response will always contain a negligible error.

To obtain the transfer function, a fast fourier transformation (FFT) is applied on the impulse response. The result corresponds to the magnitude and phase of the systems transfer function.

For a signal which is only defined for $t \geq 0$, the positive and negative parts of the spectrum are calculated by the FFT. However, as shown in figure 3.8, the dirac pulse of the autocorrelation function also contains the negative part of the time. Thus $h(k)$ is the non-causal impulse response of the system. Because of the symmetry of the FFT, only the positive part of the spectrum is calculated. This has to be considered in order to determine correctly the frequency range of the transfer function.

The main advantage of the MLS approach is speed. Since for a complete measurement only L values need to be sampled, a transfer function generally can be calculated in milliseconds. In comparison to the Lock-In this is a big improvement. On the other hand, this method has no build-in noise rejection like the Lock-In which makes it sensitive to any disturbance. An improvement can be attained by averaging the system output $y(k)$ several times, which again reduces the advantage in speed.

This measurement principle has already been developed around 1970 [38]. For a long time, it was mainly used for measurements in the area of acoustics and several publications appeared in this context [40]. Recently MLS were also used for other kinds of measurements, e.g. to measure the transfer functions of cells [8, 11].

3.3.3 The hadamard transformation

To further increase the speed of the MLS, the computational load can be reduced by using the so called hadamard transformation. This transformation can be implemented very efficiently in digital systems. In order to use it in conjunction with the MLS equation 3.26 is written in matrix form:

$$H = \frac{1}{L+1} \cdot [MY - \bar{Y}] \quad \text{with} \quad \bar{Y} = (\bar{y}, \bar{y}, \dots \bar{y}) \quad \text{and} \quad \bar{y} = \sum_{m=1}^L y(m) \quad (3.28)$$

Y is a vector containing the sampled data of the measured system. Thus $\bar{Y}$ represents the correction factor due to the DC component of the autocorrelation function. All values $\bar{y}$ in this vector are the same. M is a $L \times L$ matrix which contains the cyclically right shifted M-sequence. By multiplication with Y the correlation function is calculated like in equation 3.26. The following is a M-matrix for an M-sequence of order $n = 3$:

$$\mathbf{M} = \begin{pmatrix} -1 & 1 & 1 & -1 & -1 & -1 & 1 \\ 1 & -1 & 1 & 1 & -1 & -1 & -1 \\ -1 & 1 & -1 & 1 & 1 & -1 & -1 \\ -1 & -1 & 1 & -1 & 1 & 1 & -1 \\ -1 & -1 & -1 & 1 & -1 & 1 & 1 \\ 1 & -1 & -1 & -1 & 1 & -1 & 1 \\ 1 & 1 & -1 & -1 & -1 & 1 & -1 \end{pmatrix} \quad \mathbf{H} = \begin{pmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & -1 & 1 & -1 & 1 & -1 & 1 & -1 \\ 1 & 1 & -1 & -1 & 1 & 1 & -1 & -1 \\ 1 & -1 & -1 & 1 & 1 & -1 & -1 & 1 \\ 1 & 1 & 1 & 1 & -1 & -1 & -1 & -1 \\ 1 & -1 & 1 & -1 & -1 & 1 & -1 & 1 \\ 1 & 1 & -1 & -1 & -1 & -1 & 1 & 1 \\ 1 & -1 & -1 & 1 & -1 & 1 & 1 & -1 \end{pmatrix}$$

A order of $n = 3$ produces a 7x7 matrix. The cyclic right-shift of the M-matrix is easy to see. On the right side there is a so called Hadamard matrix. Obviously both matrices have some similarities. In the following the Hadamard matrix is used to build the autocorrelation function. This is faster than the normal computation because of an algorithm called the Fast Hadamard Transformation (FMT). It returns as a result the same as a multiplication of the matrix H by a vector would give. However, the FMT is much faster than the actual multiplication would be. Since the Hadamard matrix consists of -1 and +1 only additions and subtractions are needed. Thus the FMT is an elegant way of adding and subtracting the elements of an input vector. Often it is compared to the FFT in terms of speed. Only $O(P \cdot \log(P))$ additions are needed, where $P = L + 1$ is the dimension of the Hadamard matrix.

However, for a correct calculation, it is necessary to reorder Y before the FMT and the result thereafter. To do this two permutation matrices have to be set up. The generation of these matrices does not enlarge the computation time, since they can be calculated together with the M-sequence offline before the measurement. Thus the calculation is extended to

$$M \cdot Y = P_2 \cdot H \cdot P_1 \cdot Y \quad (3.29)$$

Furthermore an additional element has to be added to Y since Hadamard matrices are always of type $2^n \times 2^n$. Since the first column of the H-matrix is always 1, the negative DC-offset can be used as extra element. In reference [42] an example implementation of the algorithm needed to generate the permutation matrices can be found.

3.4 Noise estimation

The most fundamental limitation of every measurement system is noise. There is always a point, where a weak sensor signal is covered by the overall system noise. Hence, improving the sensitivity of the sensor requires a profound knowledge of the noise sources involved. In order to gather this knowledge one must be able to determine the noise contributions and characteristics. Therefore, this section gives an introduction on how to measure and quantify noise.

The most apparent property of noise is that it is not deterministic. Therefore, it cannot be described by a simple voltage or current value or by a function. Although the noise amplitude

is affected by fast and sudden changes, its energy is a more constant value. Thus, noise is often described by its power or more precisely by its power density. The power density $|s(t)|^2$ or $|S(f)|^2$ is defined by [13, 36]:

$$E_n = \int_{-\infty}^{\infty} |s(t)|^2 dt = \int_{-\infty}^{\infty} |S(f)|^2 df \quad (3.30)$$

Especially the spectral power density $|S(f)|^2$ is an interesting parameter. It not only describes the noise energy but also its distribution over the frequency. This is important since it further characterizes the type of noise. For example, white noise has a constant spectral power density. To measure the spectral power density, a signal $s(t)$ is sampled during a certain time T . Afterwards a FFT is computed to obtain $S(f)$. By multiplying it with its conjugated value, $S(f) \cdot S(f)^* = |S(f)|^2$ is obtained. The sampling duration T determines the lowest frequency of the FFT as $f_{min} = \frac{1}{2T}$. Accordingly, the highest frequency is fixed by the nyquist theorem as $f_{max} = \frac{1}{2} \cdot f_{\text{sample rate}}$.

However the signal spectrum is not always a useful description of noise. Especially if several signals should be compared, a single quantity is more helpful. Thus it is often favorable to calculate the SNR from the frequency spectrum. This value describes how many times the signal energy is higher than the energy of the noise. It is always advantageous to have a higher SNR since this simplifies the signal detection. The SNR is defined by:

$$\text{SNR} = 20 \cdot \log \frac{E_s}{E_n} \quad (3.31)$$

To calculate it, the energies of the signal E_s and of the noise E_n are needed. They can be extracted from the signal frequency spectrum $|S(f)|^2$. E_s is given by the value at the corresponding frequency. E_n equals the summation of the whole spectrum minus the signal energy E_s . This is a commonly used method to determine the SNR of a measured signal [43]. However, to get accurate results effects like leakage and discrete bins have to be considered. Leakage denotes that the signal energy is spread over several bins. This occurs if the sample time is not exactly a multiple of the period of the signal of interest. A similar effect appears when the ratio of the sampling rate to the sample amount is not a multiple of the signal frequency. Then there is no bin which fits exactly the frequency. Those effects can be circumvented by a correct choice of the sample parameters. However there are other approaches which use Hamming windows to provide a better solution. These are explained in detail in [44] and are not used in this work.

SNR values are important for MLS or Lock-In measurements. They describe what noise rejection is necessary to measure a transfer function with negligible error. Especially the Lock-In allows to readjust the noise attenuation. In chapter 5.2 the filter selection is discussed in the context of the noise amount which should be attenuated.

Chapter 4

Experimental setup

4.1 The amplifier

For the measurements of the microwires an analog amplifier system was custom built. It consisted of two amplification stages and an ADC which are shown in figure 4.1. Since the first stage was built as a prototype it was only laid out for one channel and all internal connections to the 16-channel sensors were pluggable. The two controls at the front allowed to set the operating point. The electronic circuit will be discussed in greater detail in subsection 5.4. The Ag/AgCl electrode (World Precision Instruments, RC4) used in this setup was immersed into the electrolyte of the chip.

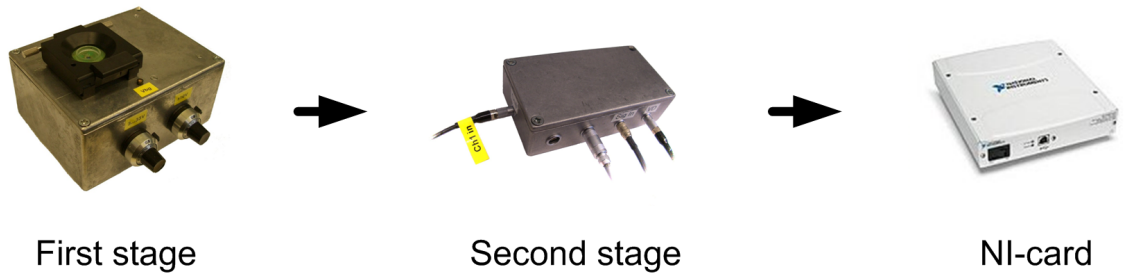


Figure 4.1: The first and second stage of the amplifier developed in this thesis and the used ADC card.

The second amplification stage was also custom built and will be discussed in subsection 5.4. To sample the analog signals an USB-6251 National Instruments card was mainly used in this thesis. It provided 16 analog inputs and two analog outputs which could be sampled with 1.25 MS/s. Some early measurements were also performed by an USB-6015 card. However this NI-card had no fast analog outputs. Thus it was not well suited for the Lock-In. As an alternative to the NI-cards a Kenwood signal generator (FGE 1202) was used in the beginning for the Lock-In development.

The Lock-In, MLS and noise computation algorithms were implemented in Labview (Labview 8.2, National Instruments). By using the DAQmx driver, the software should also work with other NI-cards. During development the software was split into several modules which should ease the reuse. Some parts of the software needed input data like M-sequences or filter coefficients, which were generated with Matlab (Matlab R14, The Mathworks Inc.).

The cell measurements in section 5.7 were performed with ISFET devices. Therefore another amplifier, the TTF-Box was used. It is shown in figure 4.2. This device can handle 16 channels in parallel. It offers a DC amplification and a 16 channels analog Lock-In, which however was not used in this thesis. At its backside it provides a connector to a USB-6251 NI-card. This connection was used in conjunction with the DC amplification. Thus the Lock-In developed in this thesis could be used for the cell measurements.

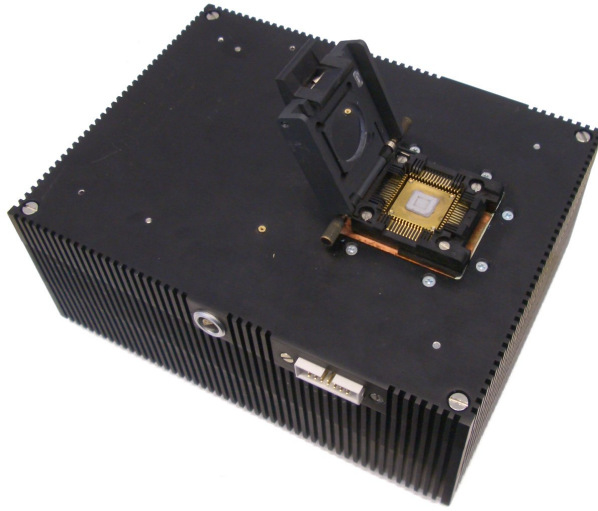


Figure 4.2: TTF-Box used for the cell measurements with ISFET devices.

Before every cell measurement, the TTF-Box was used to characterize the chips and to set the operating point. Then the electrode was connected to the output of the NI-card and the newly developed Lock-In was used. This setup allowed to automatically read out 16 channels. It is well suited for cell measurements since the TTF-Box keeps the temperature of the chip at 37 °C. In subsection 5.4.4 the electrical properties of this amplifier are shortly discussed.

Since this setup provided 16 channels, the software was extended to read them out in serial. Further software modifications were necessary since several cell experiments required to perform potentiometric and impedimetric measurements at the same time. To monitor changes, these measurements were repeated at a constant rate. However there was a bottle neck in the system setup, since only one channel could be read out at a time. Thus, to achieve high repetition rates the measurement duration had to be shortened. Therefore the transfer functions were only

recorded between 10 kHz and 600 kHz since this region showed the most changes. Furthermore to obtain the constant component the mean value was calculated from the sampled data for the Lock-In. This avoided to sample extra data and thus also shortened the measurement duration. In order to eliminate a systematic error, the sample amount was always adjusted to measure full cycles. As a general consequence the quality of the DC and the AC signals improved if a better filter was used since filters with more coefficients require more data to be sampled.

4.2 Measurement protocols

4.2.1 Electrolyte measurements

As a basic test, to show the functioning of the devices and the amplifier, transfer functions were measured at different concentrations of NaCl with microwire devices. To obtain electrolyte solutions with different salt concentrations, the corresponding amount of 1 mM crystalline NaCl was dissolved in Milli-Q water. By repeated dilution the NaCl concentration was diminished by increments of a power of ten. To verify the linear change in resistance, the conductivities of the electrolyte solutions were checked by a commercial measurement device (Mettler Toledo, SevenEasy).

Before every measurement, the microwire chips were rinsed for a minute in Milli-Q water. Thus the remaining salt on the chip was washed away. Thereafter the chips were dried with argon. To remove further impurities, the chips were regularly rubbed with a Q-Tip and ethanol. When several NaCl concentrations were measured, it was always started by the lowest one. This prevented wrong results and the chip did not have to be cleaned between the measurements. This was important since the electrolyte could be exchanged without moving the electrode position. Otherwise it would not have been possible to distinguish the influence of the salt concentration and of the electrode position. In general, 200 μl of liquid were used in each measurement.

4.2.2 Cell measurements

All cell measurements were performed with ISFET devices on human embryonic kidney cells (HEK 293) of the EAG type. Before the chips could be used, they had to undergo several preparation steps. First, the chips were cleaned and activated in order to provide a clean and non-toxic environment to the cells. Then they were coated with the cell adhesive protein poly(L)lysine (PLL). This protein layer ensured that the cells were able to tightly adhere to the chip surface. Thereafter the cells were kept for two days in a culture medium (M10) at 37 °C. For further information about the chips and preparation procedure refer to [16].

To assure a good environment for the cells, the chip temperature was kept at 37 °C during the measurements. Furthermore it was ensured that the electrolyte was isotonic and non-toxic. Depending on the measurements, the cells were immersed in different solutions like the culture medium. To measure the trypsin activity the culture medium was replaced by 150 μl phosphate

buffered saline (PBS) (see appendix D). PBS contains no calcium which would inhibit the trypsin activity. After a defined time, another 150 μl trypsin were added. This allowed to exactly monitor the signal change due to the trypsin.

In several measurements the programmed cell death (PCD) was observed, also called cell apoptosis. These measurements required a more complex buffer. Since cancer cells (HEK 293) were used the apoptosis was induced by the anti-cancer agent irinotecan hydrochloride (CPT-11). To optically verify the cell state, two fluorescence markers were added to the buffer. Annexin-V is a protein which is labeled by fluorescein (FITC). When a cell is in the early stage of apoptosis it exposes phosphatidylserine (PtdSer) at its surface to signal phagocytes, that it can be destroyed (see [45]). The annexin-V binds to these proteins and the FITC accumulates at the cell membrane. In figure 4.3(b) an example of stained cells in early apoptosis is shown. Obviously the membrane shows a green fluorescence due to the FITC. The medium also shows a slight fluorescence because of the dissolved FITC.

Besides this as second marker propidium iodide was used. In a late state of apoptosis, the membrane and nucleus become permeable for the propidium iodide. It binds non-specifically to DNA and RNA and thus can increase its fluorescence 20- to 30-fold [46]. Therefore in figure 4.3(c) only the nucleus shows a fluorescence. By superimposing the figures 4.3(a) - 4.3(c) the fluorescence could be assigned to the cells (see figure 4.3(d)). In appendix D the exact composition of the buffer solution is shown.

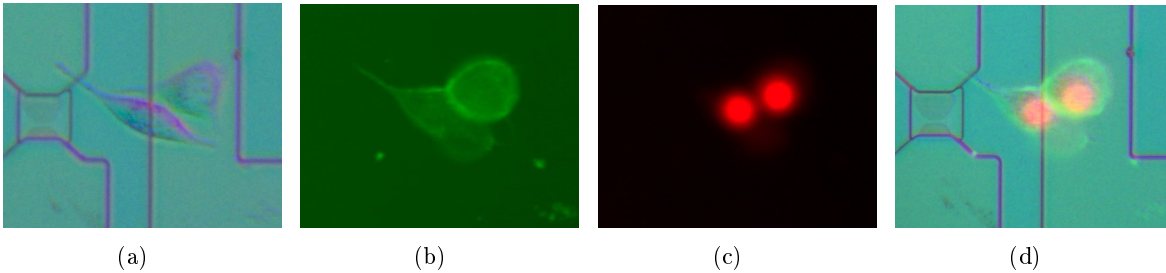


Figure 4.3: **(a)** Healthy cells on a chip. **(b)** Early stage of apoptosis marked by annexin-V. **(c)** Fluorescence of propidium iodide after cell death. **(d)** Superposition of pictures (a)-(c).

For the pH measurement, a PBS buffer with a pH of 7 was used. To adjust the pH, HCl was used to lower it and NOH to increase it. The pH was then verified with a pH meter (Mettler Toledo, SevenMulti). Furthermore to keep the specific resistance of the solution at around 60 Ωcm the conductivity was also measured (Mettler Toledo, SevenEasy).

Chapter 5

Results and discussion

5.1 Development of the Lock-In

The digital Lock-In development in this thesis consisted of a three step process. First a test implementation of the Lock-In was written in software to verify, that the system would work in principle. Furthermore it was used to generate the filter coefficients. Then the actual implementation was done, based on the results of the first simulation. In a third step the operation of the Lock-In was analyzed by comparison to a professional tool and to the simulation results. This allowed to locate error sources and to determine their influence on the results. In this section, the second development step is discussed in further detail. For more information regarding the implementation see section 4.1.

The following subsections describe problems which occurred during the development. Every subsection covers the problem source, its influence on the measurement and a work-around to eliminate the error.

5.1.1 Signal coupling

The primary goal in developing a Lock-In is to achieve a high noise rejection. In more general terms, to considerably lower signal parts in adjacent spectrum bands (in section 5.2 this ability is evaluated). Hence, when it comes to signal coupling, it seems insignificant if the signal coupling is done AC or DC. The sensors always contained a large, inherent constant component in their signal. This was due to the voltage U_{DS} which was needed to set the operating point and due to a constant drain current (see equation 5.8). In comparison to the actual signal of interest this constant component could become very large. For example, at an operating point of $U_{DS} = 1.3$ V and a high amplification, the constant drain current generated a DC component of 8 volts. At the same time the amplified AC signal had an amplitude of only 0.02 V. This would require the Lock-In to attenuate adjacent bands to over 50 dB to get rid of the DC signal. Measurements based on filters which did not fulfill this requirement showed a low pass characteristic. At low reference frequencies the filter could not attenuate the constant component because of

its magnitude. By increasing the reference frequency, the initial DC component was shifted to a high frequency by the multiplier. Until then the attenuation of the filter was not high enough to inhibit the large signal. Therefore the cut off frequency of the resulting low pass was determined by the quality of the filter. This is shown in figure 5.1.

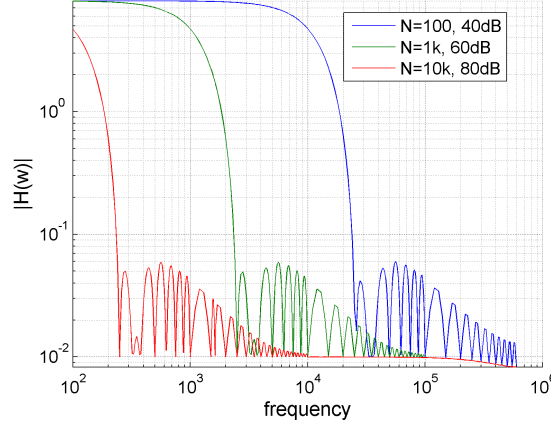


Figure 5.1: Simulated transfer functions for filters of different quality.

There is no situation in which the constant component of a signal is of interest for a transfer function measurement. Thus it was favorable to eliminate it before the signal was computed in the Lock-In. As was already mentioned before, there was also a compensation in the first stage of the amplifier. This was not only important for the Lock-In but also for the ADC to be able to use its full precision. Unfortunately, a remaining DC component was not compensated, e.g. due to changes in the sensor itself. To entirely eliminate it, the mean value was subtracted from all sampled signals.

Alternatively, a high pass filter could have been used to eliminate the DC part of the signal. However this might have caused problems regarding the measurable bandwidth. At very low frequencies the high pass would also attenuate the sensor signal. By subtracting the mean value, no frequency component was filtered out. So the measurement range was only limited by the capabilities of the DAC and ADCs. To minimize errors due to the subtraction, always multiples of one period of the signal were sampled (see section 5.1.2).

5.1.2 Measurement parameters

To achieve good measurement results over a wide range of frequencies, several parameters had to be well chosen. First of all, a filter which uses enough coefficients to attain a sufficient attenuation had to be selected. This is further discussed in section 5.2. Besides this, appropriate values for the sampling rate and the sample amount were required. This can be explained by a small example of a measurement with a maximum sampling rate of 1.25 MS/s and a filter of

20 thousand coefficients. At least as many values as the filter has coefficients must be sampled to get a correct FIR response. At high frequencies this provides satisfactory results. However at low measurement frequencies of e.g. 10 Hz sudden errors in the magnitude and phase appear. The reason is that at 10 Hz and a sampling rate of 1.25 MS/s the filter attenuation is too low. To use filters with more coefficients is not an optimal solution since this would increase the computational load unnecessarily.

In a FIR-filter the cutoff frequency is also determined by the sampling rate. This was already implied in subsection 3.2.2, where the normalized frequency was introduced. By decreasing the sampling rate, also the cutoff frequency of the low pass FIR filter is decreased. Thus it was favorable to use smaller sampling rates at low measurement frequencies. However, there was no solution on how to choose values, which work under every condition.

The Lock-In in this thesis provides several ways to solve this problem. For fast test measurements, the sampling rate and sample amount were just kept constant. Alternatively the Lock-In could calculate appropriate values for these parameters at every measured frequency. The problem was to find an approach which works under all configurations of filters and measurement ranges. A quite satisfactory solution was based on equation 5.1. It calculates the number of samples which are necessary to gather several cycles of a sinusoidal signal at a given sampling rate and reference frequency.

$$\text{sample amount} = \text{cycles} \cdot \frac{\text{sampling rate}}{\text{reference frequency}} \quad (5.1)$$

Since the reference frequency was known and the number of cycles was given by the user, the sampling rate could be calculated. As sample amount the number of filter coefficients was chosen. However it should be noted that the NI-cards did not work properly at every sampling rate. Therefore the result was rounded to a multiple of the corresponding decade. For example if a sampling rate of 213 kHz was calculated then it was set to 200 kHz.

Another problem which had to be considered were the transient effects for each frequency measurement. A certain time must elapse before the system is in a steady state. To wait for a fixed number of cycles was impractical because at very low frequencies this time would greatly prolong the measurement duration. For example at 0.01 Hz one cycle lasts over one minute, at 0.001 Hz it lasts over 15 minutes. Therefore the settling time was specified by a percental value. It defines how many extra samples should be recorded and discarded. Furthermore the resulting amount of samples was extended to ensure that always multiples of an entire sine period were sampled. By doing this, the Lock-In could calculate at the same time the constant component of the data. Therefore it calculated the mean value of all sampled data. Since it was ensured that always a full period of a sine was sampled, the sine added up to zero and no systematic error occurred.

This approach provided satisfactory results in most situations. Nevertheless sometimes artifacts remained, which were due to non-optimal values for the sample amount and sampling rate.

Therefore the Lock-In also allowed to specify these values manually for every measured reference frequency.

5.1.3 Influence of the signal source

The quality of a Lock-In is determined by the quality of its source signal. In certain aspects a Lock-In resembles a matched filter. This kind of filter is used to detect a known signal, even if it is buried under noise. Hence, this only works if the signal which should be detected is known exactly. The same problem is addressed in a Lock-In. The reference signal is the component which should be detected in the recorded data. Already small differences in the source and reference signal can decrease the detection quality. It is easy to generate a perfect reference signal, since it only needs to exist in digital form. This is not true for the source signal. It needs to be available in analog form. In order to determine the influence of the source on the measurement result, several different sources were tested.

Figure 5.2(a) shows a measurement of a sensor in a 0.1mM NaCl solution. The blue curve was measured by the SR830 and the others are based on the self developed, digital Lock-In. A low pass filter with a high and a low attenuation was used. Surprisingly the measurement based on the filter with the low attenuation showed a better agreement to the SR830. In this measurement the Kenwood signal source (see chapter 4.1) was used for the self developed Lock-In. In contrast to this, the SR830 used it's own source.

To further investigate this problem the Lock-In was fed with a fixed sine at 1600 Hz at different reference signals (more on this measuring method in section 5.2). The result is shown in figure 5.2(b).

Under ideal circumstances, there should only be a peak at 1600 Hz. Due to the limited attenuation of the filters, the width of the peak was spread. More important was the fact, that the maximum of the peak was not exactly at 1600 Hz, but slightly higher. This could only be explained, by an inaccurate signal source. Since the source did not generate exactly the frequency which was anticipated, the Lock-In regarded it as being a disturbing signal. This explains the curve of the good filter in comparison to the bad filter in figure 5.2(a). Since it had a much higher attenuation the wrong frequency decayed much faster.

The problem was solved by using the analog output of the National Instrument card as source. Internally, the same clock for the sampling of the sensor signal and for the generation of the source signal was used. This guaranteed a perfect frequency match and was used for all further measurements.

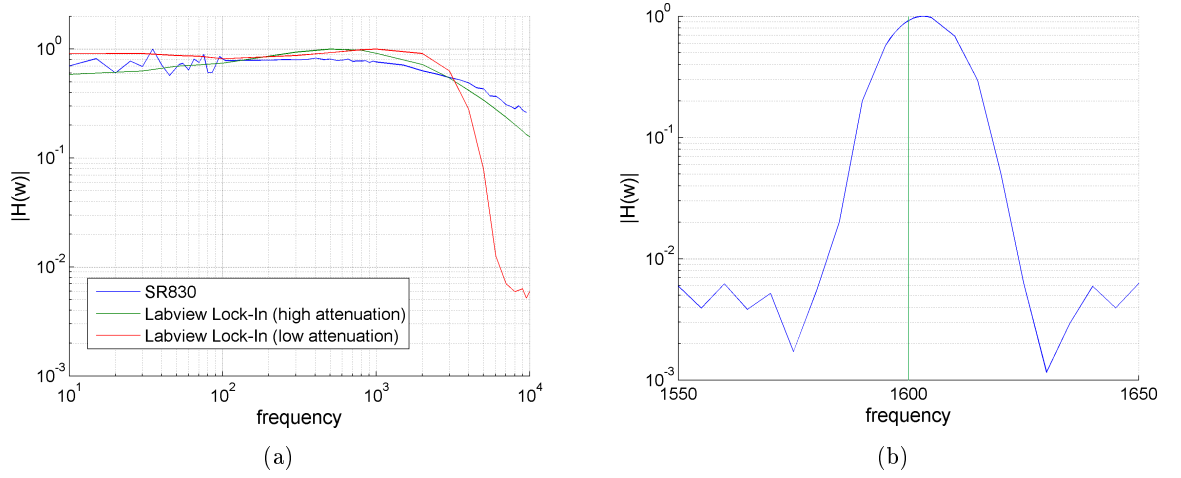


Figure 5.2: **(a)** Multiple measurements of one sensor with a good and a bad filter. **(b)** Frequency drift of the Kenwood source set to 1.6 kHz measured by the Lock-In.

5.1.4 The phase information

As it is obvious from the calculations in chapter 3.2.1, it is necessary to have a defined phase relationship between the source and the reference signal. Since synchronisation is a very common problem, there are several approaches to solve it. Some commercial Lock-Ins use a phase-locked loop (PLL) to ensure a correct phase. A PLL delays a source signal to achieve a synchronisation to an incoming signal (see [47] for more information). In this case an external PLL or an additional analog channel would have been necessary. The latter was not acceptable since it would have diminished the maximum possible sample rate, due to limitations of the NI cards.

A simpler solution was to use a trigger signal. It was generated by the source and started the data acquisition. Thus the sampling started at the beginning of the period of a sine wave. Figure 5.3(a) shows the phase measured of a sensor in 0.1mM NaCl. A problem which occurred with several different sources was a wrong triggering time. Sometimes the trigger was detected by the NI card on the falling, sometimes on the rising edge. This resulted in a sudden phase change of 180° . In figure 5.3(a) it is clearly visible, that the phase of the self developed Lock-In and of the SR830 would have a very good match by a correct trigger.

To circumvent this problem the NI card itself was used as the signal source. As mentioned in subsection 5.1.3 this allowed to sample and generate a signal by using the same clock. Not only the frequency was exactly the same, but this also allowed an exactly fixed phase relationship. Unfortunately, this did not prevent errors. Even though the NI card was synchronized internally, there was a small trigger delay remaining. At high frequencies the delay can add a linearly increasing phase error. This is due to the higher influence of one sample point to the phase. For example, consider a sample rate of 200 kHz and two sampled signals at 10 Hz and 10 kHz,

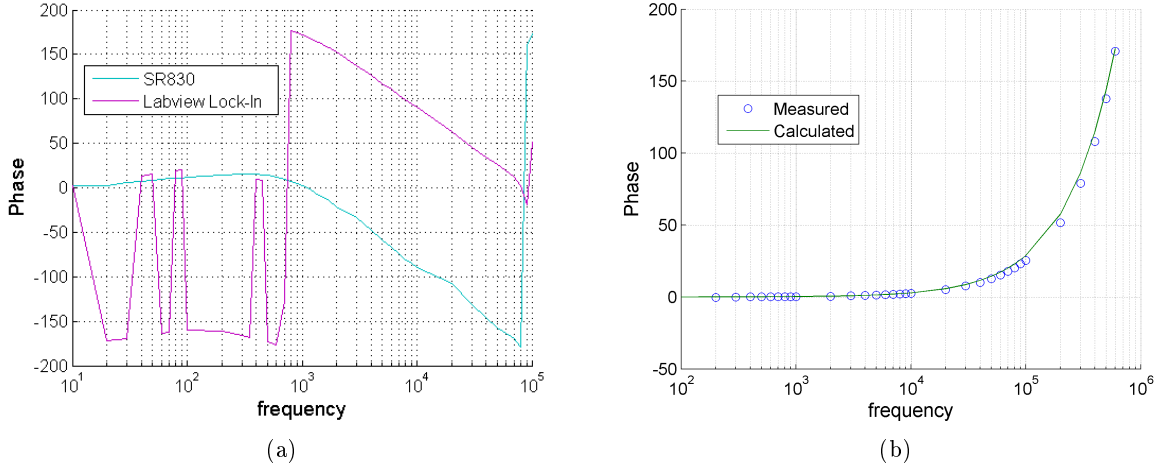


Figure 5.3: **(a)** Correct and disturbed phase by an unstable trigger. The unstable trigger causes a temporal phase shift of 180°. **(b)** Error in phase information due to a constant trigger delay.

respectively. In the first case, if the signal is delayed by one sample due to the incorrect trigger, this results in a additional phase error of 0.018°. In contrast at 10 kHz it equals to 18°. For higher trigger delays the phase error even increases further. Figure 5.3(b) shows an example of such a measured error. The error can simply be calculated by:

$$\text{Phase error} = \frac{360 \cdot \text{reference frequency}}{\text{sample rate}} \cdot \Delta n_{\text{samples}} \quad (5.2)$$

$\Delta n_{\text{samples}}$ is the trigger delay given in missed samples. Since the trigger delay always seemed to be constant, the digital reference signal was delayed accordingly. Aside this discussion it should be noted that all these errors do not influence at all the magnitude of the transfer function. This is comprehensible since it does not contain any phase information.

5.2 Lock-In benchmark

As was already mentioned earlier the most important feature of a Lock-In is a good noise rejection. This describes the ability to attenuate strong signals in adjacent frequency bands and determines how large the noise signal can be without adding a considerable error to the output. The higher the attenuation the larger the noise component can be. In this section some definitions regarding noise rejection are introduced. Furthermore the properties of the self developed Lock-In and the SR830 are compared directly.

5.2.1 The dynamic range

An important specification of an amplifier is its dynamic range. The dynamic range of a Lock-In describes the maximum noise at which a measurement is still possible. It is defined by the ratio of the largest and smallest allowed signal. Generally the upper limit is defined by the point when the amplifier saturates. The lower limit could for example be the weakest possible signal which can be detected. Usually the dynamic range is given in decibel:

$$\text{Dynamic range} = 20 \cdot \log \left(\frac{V_{\max}}{V_{\min}} \right) \quad (5.3)$$

In an analog Lock-In the dynamic range is an important point to consider. It can happen that a component in the signal path like the multiplier, low pass filter or DC-amplifier reaches its saturation point. The trigger could just be the high level of input noise, which is often much bigger than the signal of interest. In such a case the output signal is not correct anymore. To overcome this problem the overall gain in an analog Lock-In is distributed over several stages. At the input stage mostly a low amplification is used, to prevent saturation of the input amplifier by high noise. However, the multiplier behind the input amplifier needs a certain minimum voltage to operate. Thus the input signal is limited to an upper and lower limit and the dynamic range is fixed by equation 5.3.

In a digital Lock-In only the ADC can saturate. After the digitization all calculations are performed with double precision. This makes a saturation very improbable. The NI 6251 sampling card used in this thesis, had a 16 bit ADC. Thus the minimum detectable signal would have an amplitude of 0.3 mV peak-to-peak if the ADC has an input range of ± 10 V. If this signal is disturbed by a noise component with the maximum amplitude of ± 10 V, the noise would be stronger than the signal by

$$20 \cdot \log \left(\frac{20V}{0.3mV} \right) = 20 \cdot \log(2^{16}) = 96.3db \approx 100db \quad (5.4)$$

Already slightly higher noise would saturate the ADC and a signal processing would be impossible. Thus the Lock-In has a dynamic range of 100db. As such it can even detect a signal if the noise component is 100 dB times larger than the actual signal.

5.2.2 The dynamic reserve

Manufacturers of commercial Lock-Ins provide the dynamic reserve [48] to specify their noise rejection ability. It is comparable to the dynamic range but sets a more challenging requirement to the Lock-In. More precisely, it specifies how large an interfering signal at a discrete frequency can be, before the output of the Lock-In is significantly disturbed. Generally, all noise which produces an output error below 5% is regarded as being tolerable. This is a stronger constraint than just specifying the point where the Lock-In would saturate, as it accounts for the dynamic range.

The dynamic reserve is strongly related to the attenuation of the filters. The higher the attenuation, the lower the output error will be. It is important to note, that the attenuation is a frequency dependent value. In case of the low pass filters used in the Lock-In, the higher the frequency the more the signal is attenuated. Thus not only the absolute attenuation value is of importance, but also the slope. For those reasons also the dynamic reserve is a frequency dependent value. It is defined as the ratio of the maximum noise and signal amplitude under the constraint of a 5% error. As an example the attenuation is calculated to achieve a dynamic reserve of 100db. This is the maximum value of the Lock-In since it is limited by the dynamic range (see chapter 5.2.1). It is assumed that the error due to the noise is simply added to the correct signal. The attenuation A should be so large that the remaining noise is 5% of the signal amplitude:

$$\frac{V_{\text{noise}}}{A} = V_{\text{signal}} \cdot 0.05 \quad \Rightarrow \quad A = 20 \cdot \log \left(\frac{V_{\text{noise}}}{0.05 \cdot V_{\text{signal}}} \right) \quad [db] \quad (5.5)$$

The ratio of the noise to signal amplitude can be replaced by the required dynamic reserve. By using equation 5.5 the attenuation is calculated as:

$$A = 20 \cdot \log \left(\frac{V_{\text{noise}}}{V_{\text{signal}}} \right) - 20 \cdot \log(0.05) = 100 \text{ db} - (-26 \text{ db}) = 126 \text{ db} \quad (5.6)$$

In the case of the system developed in this thesis, this implies that filters are needed with an attenuation of 126 db to have a correct output for the worst condition of noise. This leads to the conclusion that a higher attenuation would be of no use, since an error of 5% is regarded as being negligible.

5.2.3 Validation of the Lock-In

To compare the self developed Lock-In to the SR830 the dynamic reserve would be suited best. However, the attenuation can be measured easily and is proportional to the dynamic reserve (equation 5.5). So only the attenuation abilities of both amplifiers were compared.

The attenuation was measured by a simple approach. The Lock-In input was fed by a perfect 2 Vpp sine wave at 1600 Hz. At the same time the reference frequency w_{ref} was swept over the measurement range. A perfect Lock-In should only measure a peak at 1600 Hz. At all other frequencies, the signal should be attenuated to zero. However, since the attenuation of the filter is limited, the peak is broadened and even for frequencies far from the reference the measured signal is not removed completely. This kind of measurement simulates a discrete interfering signal as mentioned in the dynamic reserve definition. In figure 5.4(a) such a measurement, with the SR830 is shown. The higher the filter slope of the SR830 was selected, the sharper the peak became. The only maximum was at 1600 Hz, when the reference frequency matches the input signal.

The Lock-In developed in this thesis provided two types of filtering:

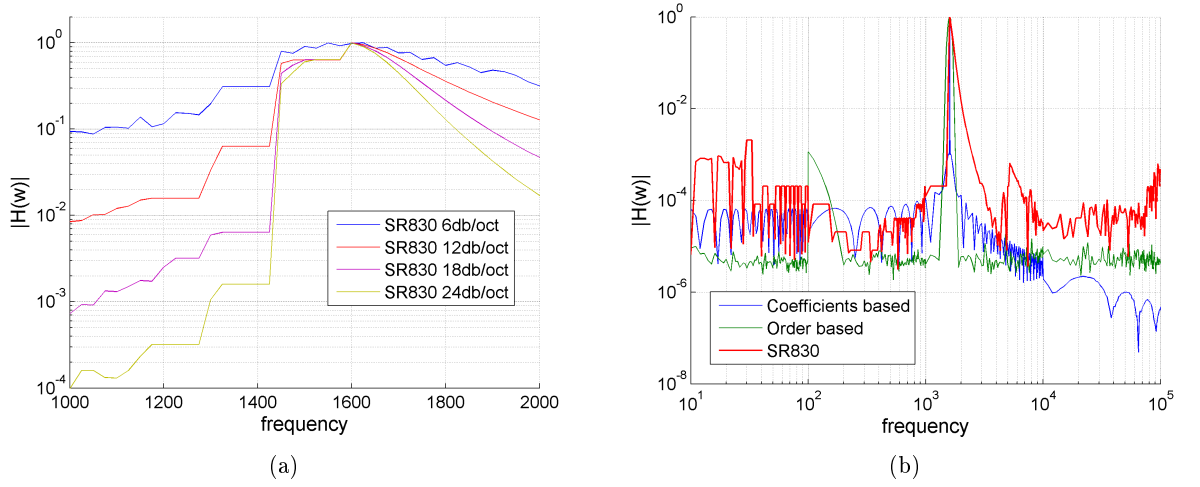


Figure 5.4: **(a)** Frequency dependent attenuation for different filter slopes of the SR830. **(b)** Attenuation comparison of SR830 at 24db/oct and the self developed Lock-In.

- In one mode, the whole filter response was calculated, as it was discussed in subsection 3.2.2. This had the advantage that the filter response showed clearly if all disturbing components of the signal were attenuated. If the filters were not chosen adequately the filter response was not constant. Furthermore the attenuation could be increased by adjusting the order. A disadvantage of this method was the high computational load of this filter. If a filter of order n with c coefficients and an amount of data of d values was used, it was necessary to calculate at least $O(n \cdot c \cdot d)$ floating point multiplications. Furthermore with $d \geq c$, the best case would have been $O(c^2 \cdot n)$ operations. However as the measurements in the next paragraph show, at least 10.000 coefficients should be used for a filter to get a good attenuation. This would require at least 10 million operations for each frequency.
- In another approach, only the first stationary value of the filter was calculated. This corresponded to the dot product of the data and the filter coefficients. So every measured point only needed $O(c)$ floating point operations. Since no filter response was actually calculated, the filter order could not be increased. The only way to increase the attenuation was by using more coefficients. Nevertheless the results were computed much faster.

In figure 5.5 both kinds of filtering are compared. For figures 5.5(c) and 5.5(d) the whole filter response is calculated while figures 5.5(a) and 5.5(b) show the fast computed case. In both cases the figures on the left side were actually measured with the Lock-In while the ones on the right were simulated.

It is easy to see in figure 5.5(d) that an increase of the filter order significantly improved the attenuation. However, this was only the case for the simulated curves. There was no improvement in figure 5.5(c) for $n \geq 2$, because of the noise of the NI-card. Beside a large signal at 1.6 kHz, there was a noise component from the NI-card spread over the entire frequency range.

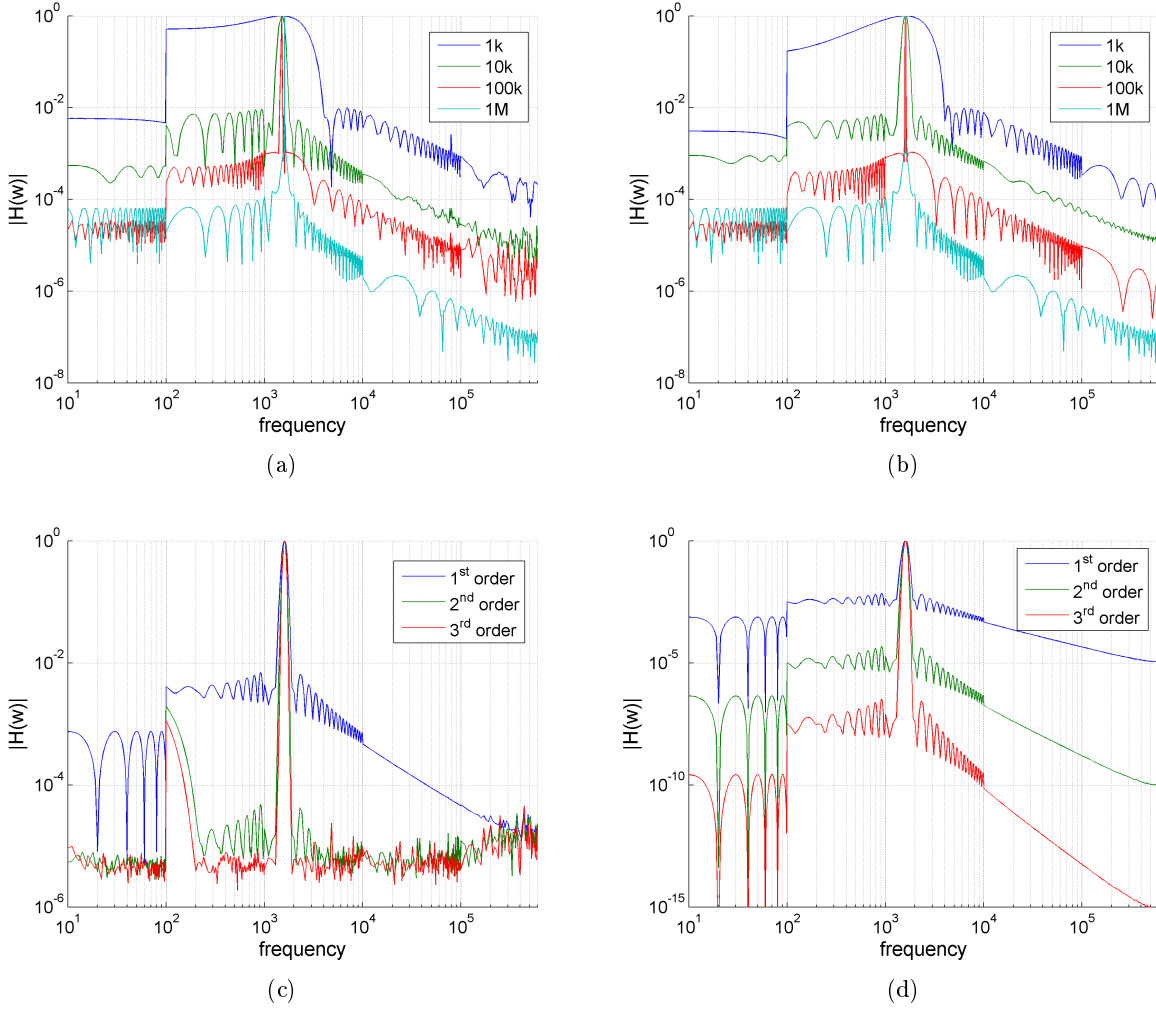


Figure 5.5: Attenuation of the Lock-In for coefficient based filtering: **(a)** Measured and **(b)** simulated. Attenuation of the Lock-In for order based filtering (10k coefficients). **(c)** Measured and **(d)** simulated.

Since at the reference frequency every signal was not attenuated at all, this noise was visible at the output. By further increasing the order of the filter only the noise floor of the NI-card was measured. Surprisingly, this was no problem for the coefficient based filtering. The reason was the increasing slope of the filters. It can be seen in figure 5.5(a) and 5.5(b) that with increasing number of coefficients, the peak becomes much sharper. If a filter with a higher number of coefficients had been used in figure 5.5(c), the noise floor would be smaller because less noise could contribute to the result.

For most measurements with biomolecular sensors, a filter with $1 \cdot 10^4$ coefficients was enough.

However, in some cases a filter with $1 \cdot 10^6$ coefficients was necessary. In general the results showed that the attenuation could be adjusted easily. To choose the best filter for a measurement was always a bit of trial. In figure 5.4(b) the attenuation is finally compared. It is apparent that the newly developed Lock-In can compete with the SR830.

Finally a comparison was made between the self developed Lock-In and the SR830. As a test system a simple electric low pass was used ($R = 100 \text{ k}\Omega$, $C = 10 \text{ nF}$). In figure 5.6 the transfer function of the low pass, measured by the self developed Lock-In and the SR830 can be seen. Both measurements show good agreement, with only a small error for high frequencies. This might be due to the different sampling rates and as such to the different measurement ranges.

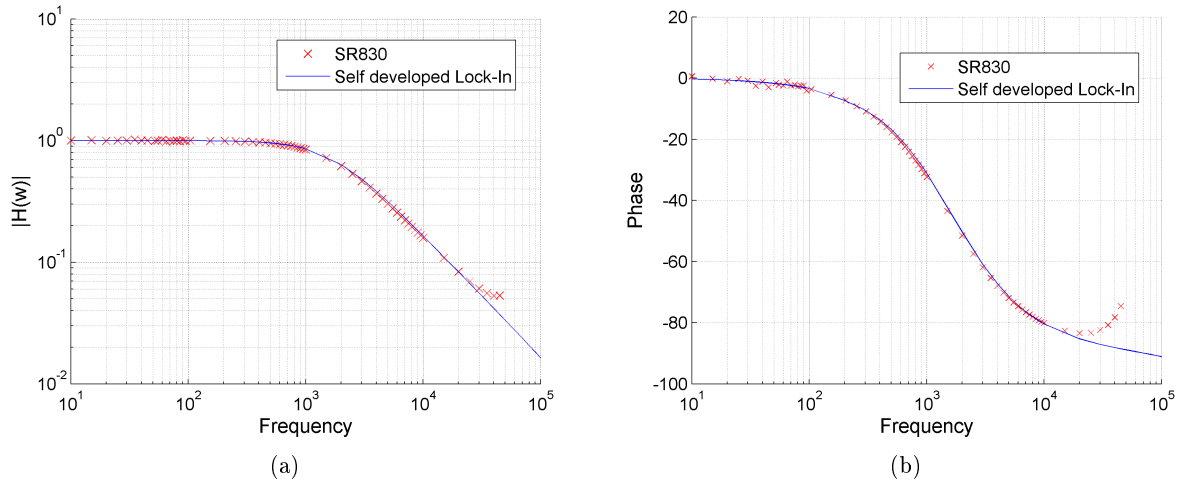


Figure 5.6: (a) Magnitude and (b) phase of the transfer function of a passive RC-low pass measured by the self developed Lock-In and the SR830.

5.3 MLS development

To adjust a MLS measurement only the order of the M-sequence, the sampling rate and the number of averaging steps can be changed. The sampling rate determines by the Nyquist sample theory the highest frequency, which can be measured correctly. By the MLS order the lower frequency limit is fixed. A longer sequence results in a longer sampling time and so lower frequency components can be considered. Therefore the measurement range is given by:

$$f_{\min} = \frac{f_s}{2^n} \quad f_{\max} = \frac{f_s}{2} \quad (5.7)$$

Since the FFT spreads the frequencies equally between $f_{\min}$ and $f_{\max}$, one receives $2^n - 1$ frequencies at multiples of $f_{\min}$.

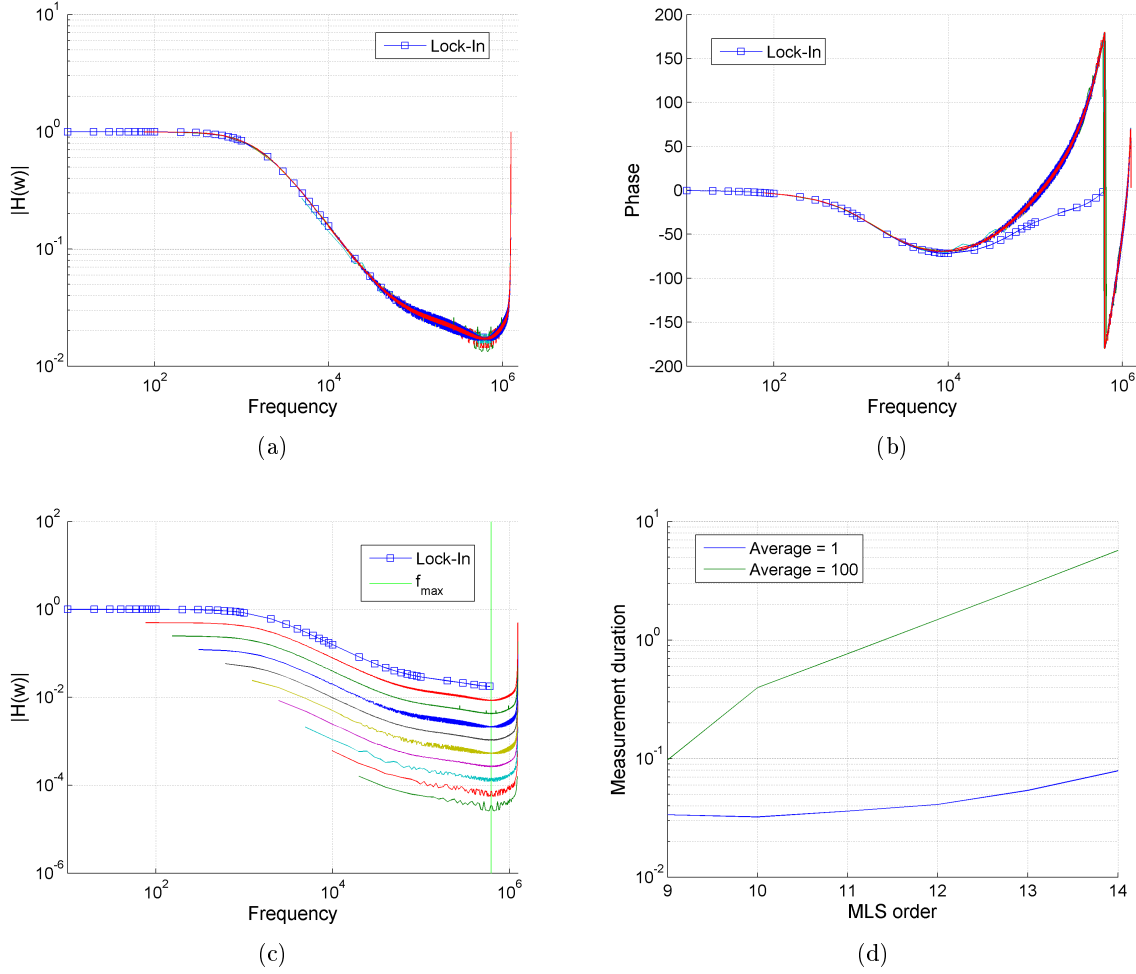


Figure 5.7: **(a)** Magnitude and **(b)** phase of transfer functions measured by MLS systems of the order $n=6..14$. The blue curve is a control measurement done by a Lock-In. In figure **(c)** the curves of (a) are shifted vertically to show the changing measurement range. **(d)** Duration of MLS measurements in seconds for different noise rejection and M-sequence length.

In order to verify the MLS measurements they were compared with the self developed Lock-In. In figure 5.7 the results are shown. The transfer function derives from a low-pass, consisting of a resistance of 100Ω and a capacitance of $10 \mu F$. Figures 5.7(a) and 5.7(b) contain the curves from MLS measurements of order 6 to 14. All measurements have been averaged 10 times to exclude any noise influence on the quality of the results. An additional measurement was done by the self developed Lock-In. It is highlighted by blue square markers.

The MLS measurements showed a very good agreement with the Lock-In results, especially

for the magnitude. Even when the MLS order was decreased there was no significant additional error. However, by decreasing the MLS order, the measurement range diminished as described above. To demonstrate this, figure 5.7(c) shows the same results for the magnitude, but the curves are shifted to better show their differences. Furthermore the maximum frequency, defined by the Nyquist theorem, is marked. Those measurements were done at a sampling rate of 1.25 Mhz. Thus all data above 625 kHz should be regarded as being incorrect.

The phase showed a considerable error starting at approximately 10 kHz which was independent from the MLS order. To avoid this error an increase of the sampling rate would be necessary, as was already shown in [8].

Finally the measurement duration for different configurations was recorded and plotted against the MLS order (see figure 5.7(d)). Obviously by increasing the MLS order and the average cycles the measurement duration increased. Thus if a large amount of measurements should be done with the MLS approach (for example of a large sensor array) it seems favorable to first do a test measurement. This could be compared with the Lock-In results to find out the best parameters in terms of frequency range and noise rejection. Assuming an array of 4096 sensors and the extremums of figure 5.7(d), this would result in measurement times ranging from 2 minutes to 6 hours.

5.4 The analog amplifier

An amplifier should provide a good signal enhancement. At the same time it should not degrade the signal by addition or attenuation of any signal parts. This is important to ensure that all measured effects are only due to the sensor and not to the measurement system itself. In this section, the analog parts of the amplifier are analyzed with the tools developed in this thesis. Different error sources caused by the analog components are shown. By variation of circuit elements, a satisfactory amplification solution is found.

5.4.1 The source setup

As it was already mentioned before, the signal source is crucial for the quality of the measurement, which accounts not only for Lock-In measurements. Generally an imperfect source signal cannot be corrected later in the signal path. Therefore it is advisable to put some effort into signal generation.

The best signal source turned out to be the DAC of the National Instrument card itself. Since the signal generation and sampling could be synchronised internally, it guaranteed a high frequency stability for the Lock-In. Furthermore it provided a high degree of freedom, what kind of signal should be generated. This was important for the MLS measurements, where a noise-like signal was necessary.

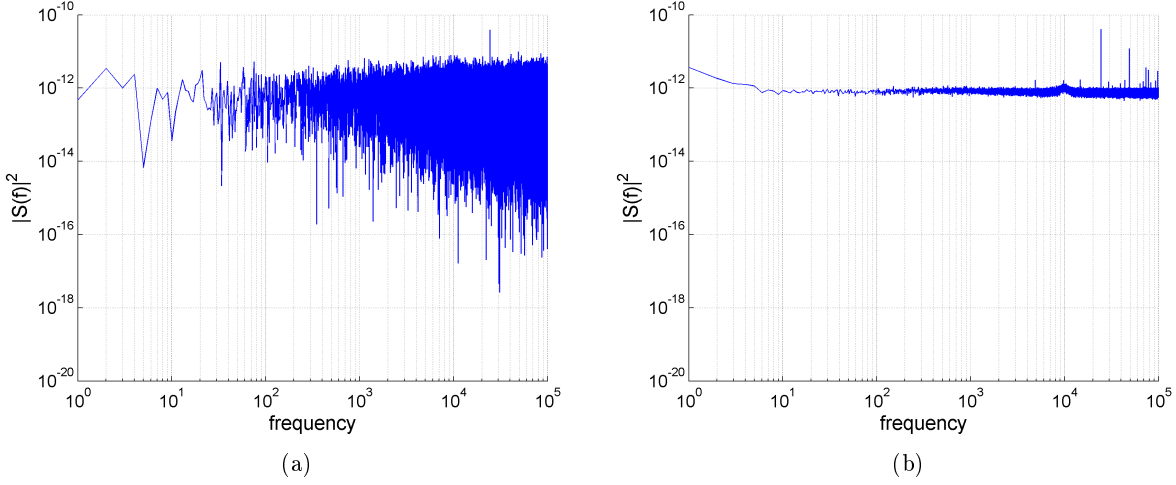


Figure 5.8: Comparison of power density spectrum measurements done **(a)** without and after **(b)** 100 averages.

To show the performance of the source, the spectral power density was measured as described in section 3.4. In order to obtain a good estimation of the spectrum, the measurement was averaged 100 times. This was necessary since noise is a statistical process as demonstrated in figure 5.8(a) and 5.8(b). In this case the DAC was directly connected to the ADC. Since no signal was applied to the DAC, only the noise floor should be measured. In figure 5.8(a) without averaging, the power density shows strong variation over the frequency. An average of 100 times in figure 5.8(b) smoothes the power distribution and reveals more spectral components which were hidden in the variations before.

The same measurement as in figure 5.8(b) was done for a sine wave at 1 kHz and 100 kHz. Figure 5.9(a) shows the corresponding spectra. To be able to better distinguish both spectra the green curve was shifted vertically by $1 \cdot 10^{-5}$. There are high peaks at 1 kHz and 100 kHz respectively, which correspond to the sine waves. The amplitude of both sine waves was $V_s = 10$ mV, which is generally also used as input for the sensor characterization. At a higher amplitude, the approximation of a constant transconductance g_m would not apply any more. Therefore the source should work best in the mV range. An amplitude of 10 mV should result in an energy of $E_s = \frac{1}{2} \cdot V_s^2 = 5 \cdot 10^{-5}$ W, which is in good agreement with figure 5.9(a).

The quality of the signal was very good, since it was 10^8 times bigger than the noise floor. However at frequencies above the sine wave there were several additional components which decreased the SNR of the source. These were harmonics of the sine wave, since they appeared at discrete multiples of the signal frequency. Furthermore they were located at different frequencies depending on the frequency of the input signal (compare the spectra in figure 5.9(a)). The harmonics were caused by limits of the DAC. At an amplitude of 10 mV, only 64 discrete

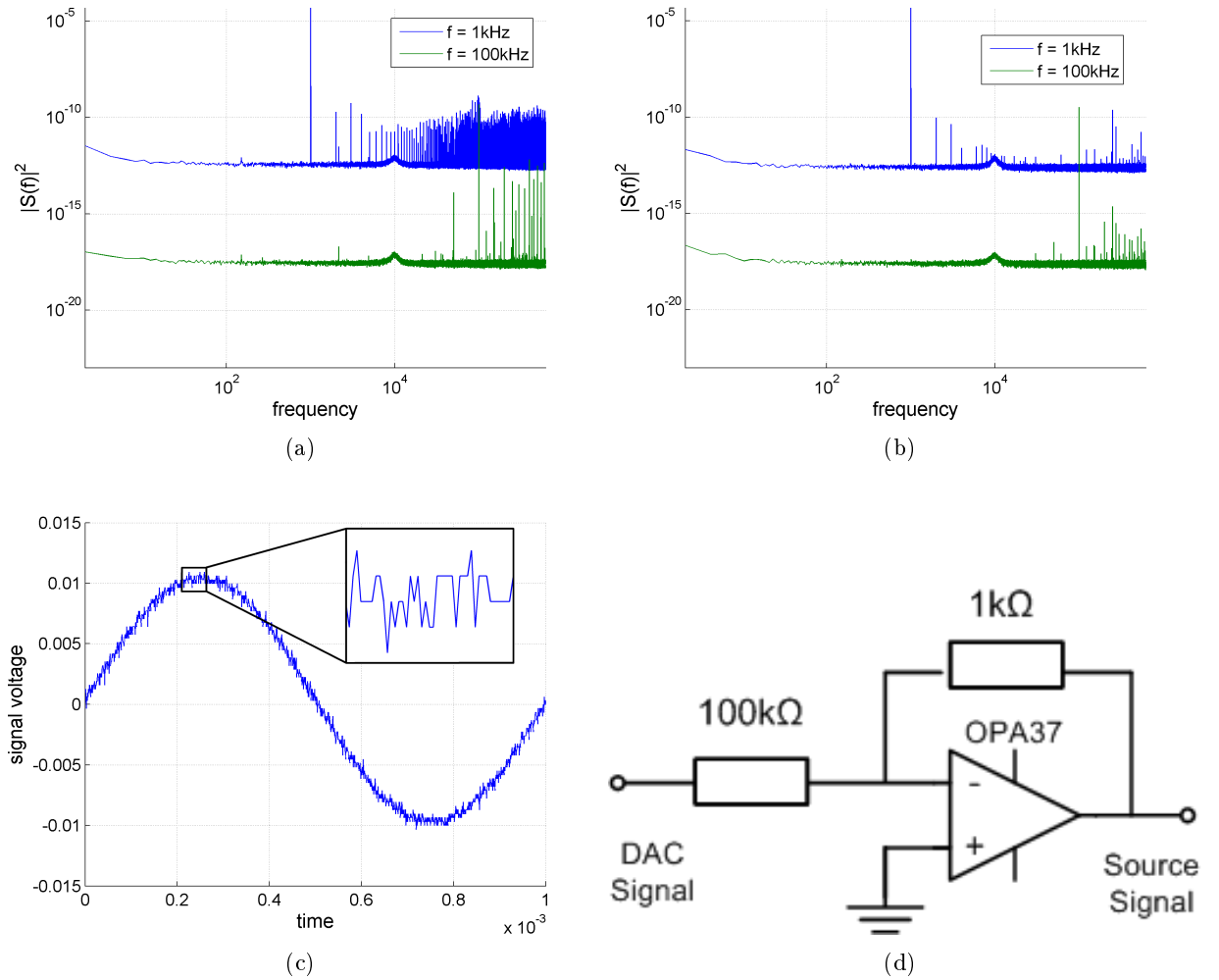


Figure 5.9: (a) Spectral power density of a sine wave at 1 kHz and 100 kHz. (b) The same spectrum after the signal has been lowered from 1 V to 0.01 V by a voltage divider. (c) Example of a sine wave with an amplitude of 10 mV, which is directly generated by the DAC. (d) Circuit used to improve the signal quality.

voltage steps remain to generate the signal with 1250 sampling points per period. Especially for low signal frequencies and high sample rates the DAC lacks steps in between to generate a clean sine wave. As an example, one period of a sine wave at 1 kHz and 10 mV is shown in figure 5.9(c).

This problem was solved by using a voltage divider. The signal was generated at an amplitude of 1 V and reduced afterwards to the desired voltage. Therefore, the DAC could insert more steps in the sine wave and the harmonics were attenuated. This can be clearly seen in figure 5.9(b). A voltage divider of 1 to 100 showed the best results. However, even if this improves the

measurements of the spectrum, it should have no influence on the Lock-In. Since the harmonics were generally five decades smaller than the signal, the Lock-In should attenuate them easily.

Actually, first Lock-In measurements of a passive low pass with the improved source showed a shift of the transfer functions to lower frequencies. The reason was the changed impedance of the source. Normally the DAC had an output impedance of $0.2\ \Omega$. The voltage divider significantly increased this impedance. As it will be shown later, the measured system can be modeled as a passive network of resistances and capacitances (see section 5.6). To ensure that the properties of the source have absolutely no influence on the measurements, a circuit as shown in figure 5.9(d) can be used.

This circuit is an inverting amplifier. Its resistances were chosen to attenuate the signal by a factor of 100. Unfortunately the signal was also inverted by the circuit. In order to measure the correct phase information, the source signal should be inverted in the software which is easy to achieve. Measurements based on the Lock-In showed that the additional operational amplifier did not change the transfer function. Also the power density spectrum was very similar to figure 5.9(b), but with the addition of a slightly higher noise floor. Nevertheless the SNR was slightly improved to a value of 26.9 dB from 25.5 dB without the voltage divider. Therefore this improvement was most interesting, if the noise spectrum was analyzed or if the sensor impedance needed to be decoupled from the measurement system. Other measurements methods like the Lock-In should not have benefited from this, since the SNR improvement was too small.

There were some remaining peaks above 10 kHz which already showed up in figure 5.8(b). These could be associated partly to the DAC and ADC. However because of their size they were negligible and should have had no influence on any kind of measurement.

5.4.2 Transfer function of the ADC

Besides the peaks mentioned in the chapter before, the NI-card had a slight influence on the Lock-In measurements. In figure 5.10(a) and 5.10(b) the transfer function of the NI-card is shown, when the DAC was directly connected to the ADC. Ideally the magnitude would have always stayed at one, with a constant phase of 0° . However there was a small error for high frequencies. If the Lock-In output was directly connected to its input without transformation to an analog signal this error disappeared. Therefore it must have been due to the NI-card. However in most cases it should have been neglectable. Furthermore, it seemed invariant to all parameter changes in software. Therefore the measurements could be corrected by dividing the magnitude and subtracting the phase from the measured data shown in figures 5.10(a) and 5.10(b).

5.4.3 Design of the amplifier

The primary goal of an amplifier is to increase the signal amplitude with a minimum of additional error sources. In figure 3.1 the general amplifier concept was already shown. In this chapter this

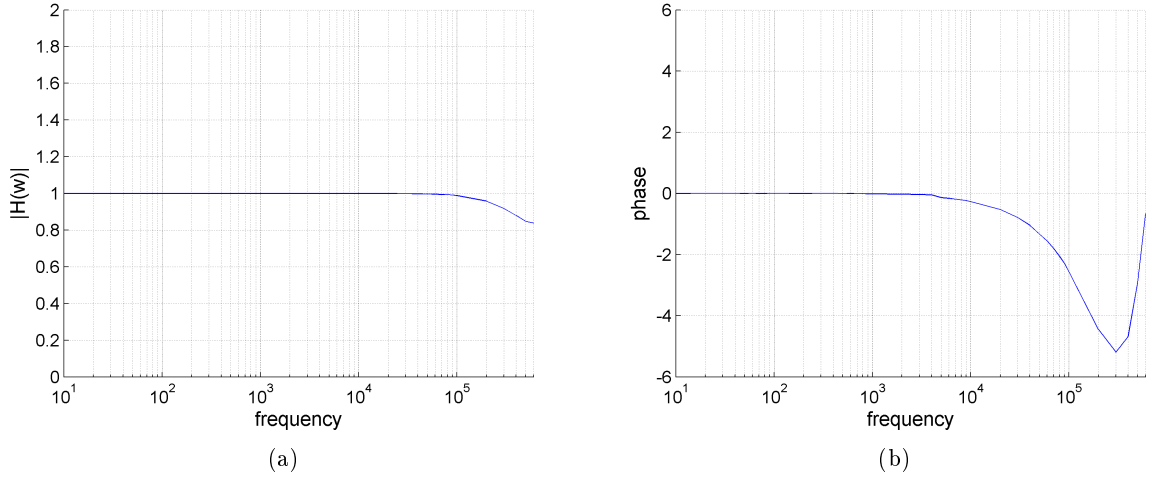


Figure 5.10: **(a)** Magnitude and **(b)** phase of the NI-card transfer function.

concept is explained in greater detail. The goal was to design an amplifier which influences the measured signal as little as possible. However there was always a trade-off between gain and bandwidth. Therefore two approaches were used. The first approach distributed the gain of the amplifier over several stages, while the second only used one stage of high quality. Both amplifiers were verified with the tools developed in this thesis.

The distributed gain approach consisted of a first and a second amplification stage. The first stage converted the current of the sensor into a voltage signal, applied the operating point of the sensor and performed a first signal amplification. This was done by an impedance converter as shown in figure 5.11(a). The terminal I_D was connected to the drain of the sensor. U_D was the drain voltage which was used to set the operating point of the sensor. The output voltage $U_{O_{ut}}$ of this circuit is calculated by:

$$U_O = U_D + R_A \cdot I_D \quad (5.8)$$

Obviously R_A is the amplification factor of this circuit while U_D is an offset for the output voltage. In order to work correctly the operational amplifier must have a very high input impedance. This was important since the sensor wires have a high resistance of around $10^6 \Omega$. If the input resistance of the operational amplifier is not considerably higher, it would cause additional currents which distort the measurements. In this thesis a TL071CP was used with an input resistance of $10^{12} \Omega$.

The second stage has a more complex circuit as shown in figure 5.12. In principle it can be separated in three sub-circuits. The first part has two input signals. U_{in} is the output signal of the first stage and contains the actual sensor signal. U_{comp} is a compensation voltage, which is set by the national instruments card. As equation 5.8 shows, the output of the first stage does

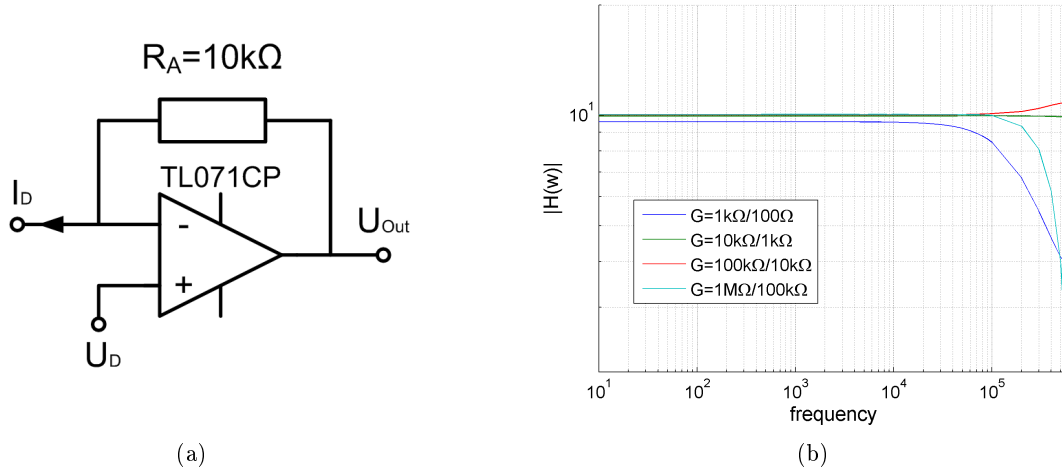


Figure 5.11: (a) Circuit of an impedance converter. (b) Transfer functions for different amplifications of an inverting amplifier with an OPA37.

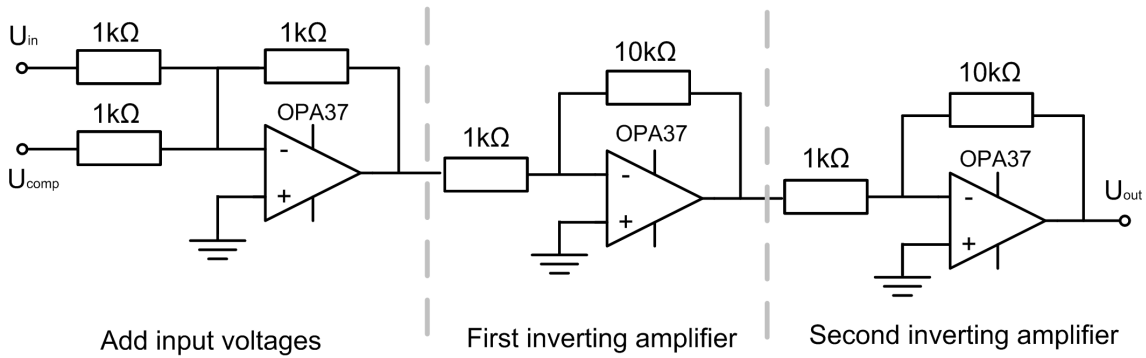


Figure 5.12: Second stage of microwire amplifier.

not only consist of the sensor signal $R_A \cdot I_D$ but additionally contains the operating point voltage U_D . The higher this operating point voltage U_D is, the sooner the amplifier will saturate. To avoid this problem it is subtracted by the first sub-circuit.

Thereafter, the remaining signal is amplified by two inverting amplifiers. They are realized by two OPA37 which are very fast operational amplifiers. If the OP is too slow, the gain will strongly decrease at high frequencies. Thus no information about the sensor above this frequency can be deduced. Beside the question which OP to use, the dimensioning is also important. In figure 5.11(b) the transfer function of one inverting amplifier is shown. All curves are for a gain of 20 dB, but the coupling resistances are dimensioned differently. An ideal amplifier would show a perfect flat magnitude over all frequencies. This is the case for the combination of 10 kΩ/1 kΩ. In fact all three sub-circuits could have been combined in such a way, that only one OP would

have been necessary. However then it would show a cut-off frequency in the measurement range, which should be avoided.

In contrast to the first stage, the input resistance of the OP is not important here. The signal is decoupled from the sensor due to the low-ohmic source of the first stage.

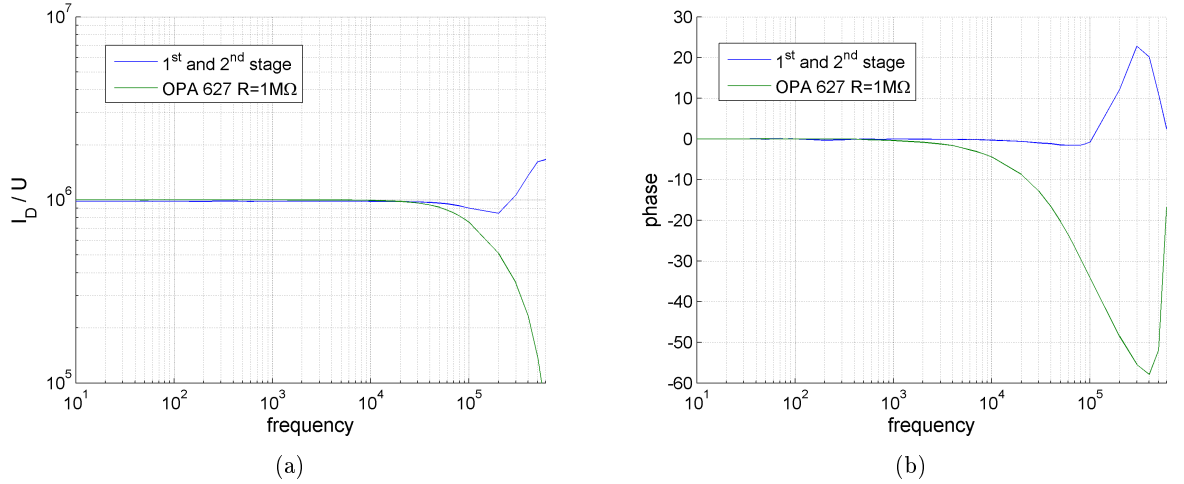


Figure 5.13: **(a)** Magnitude and **(b)** phase of the two stage and the OPA627 amplifier for the microwire devices.

In principle the amplifier could have been realized just by the first stage. If R_A would be in the mega-ohm range the output signal would be large enough for the NI-card to be detected. Thus there would be no need for a second stage. However a high R_A and as such a high gain decreases the bandwidth of the amplifier [13], which should be avoided. Nevertheless to increase the cut-off frequency very fast OPs must be used, which in our case also must have a high input resistance. Such OPs are very rare and as such very expensive, e.g. the OPA 627. It offers an input resistance of $10^8 \Omega$ and a cut off frequency above 1 MHz.

In order to analyze which approach leads to better results, the transfer functions of both amplifiers are compared in figure 5.13. As input signal a shunt resistance of $1 M\Omega$ is used to generate a reasonable sensor current. Obviously both approaches are not ideal. Even though the two stage amplifier shows slightly better results it also required a higher building effort. In contrast the OPA627 generates a larger error in the transfer functions, but it requires less circuitry.

Since the curves in figure 5.13 should always remain constant, they can be used to correct the sensor transfer functions. However the shunt resistance, which was used for these measurements, does not provide an ideal current source. When the OP reaches its cut-off frequency, then it cannot keep its inverting input at a constant voltage. This will alter the current of the shunt resistance and give misleading results. Therefore a compensation of the amplifier influence on

the transfer function can only diminish an existing error.

Beside the transfer function also the noise characteristic of the amplifier was measured. Therefore the SNR of the source and at the amplifier output was recorded. Since the signal and the noise component of the input signal are amplified the SNR can only become worse. However measurements showed that the SNR values became better. The reason is the cut-off frequency of the amplifier. Since noise at high frequencies is attenuated, the SNR seems to improve. Thus it only makes sense to measure the parts of the spectral power density where the transfer function remains constant. In this range no significant changes in the SNR could be detected. In section 5.5, measurements of the sensor noise will be presented. In comparison to the amplifier noise, this noise source is much larger.

5.4.4 TTF-Box characteristics

In section 5.7 several measurements will be described, which have been performed with the IS-FET TTF-Box. As was already mentioned, the TTF-Box was connected for these measurements to a NI-card which sampled the data. The computation was performed by the self developed Lock-In. Internally the DC amplification path of the TTF-Box was used. However this signal path was only dimensioned to amplify the constant signal component. Therefore at high frequencies the electronics are not optimal. To clearly separate their influence from the sensor information, the transfer function of the TTF-Box was measured. The sinusoidal input signal was fed over a $5\text{ k}\Omega$ shunt resistance into the amplifier. In figure 5.14 the magnitude and phase of the TTF-Box transfer function are shown. All TTF-Box measurements in the following chapters are corrected by these curves.

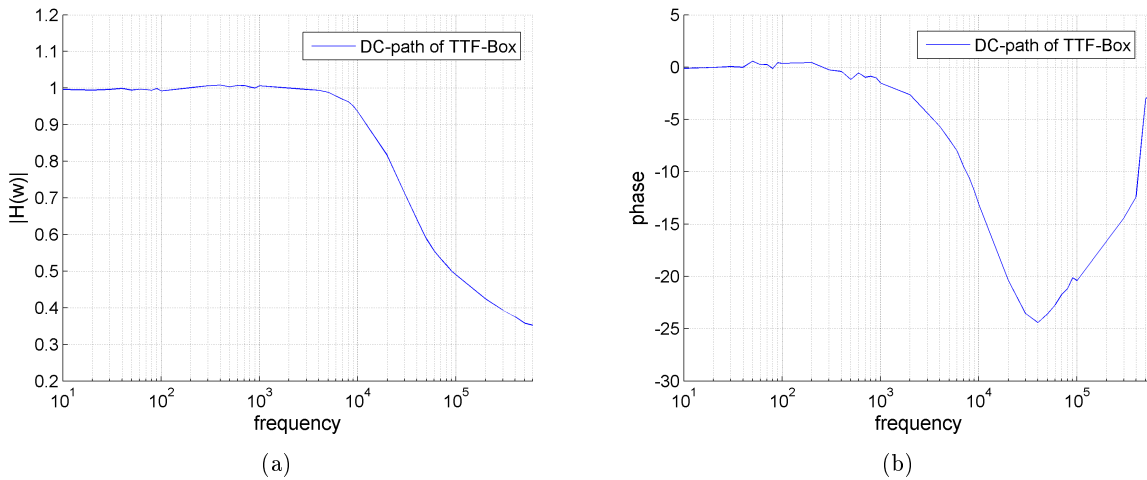


Figure 5.14: Magnitude **(a)** and phase **(b)** of the DC amplification path of the TTF-Box.

5.5 Noise characteristics of ISFETs and microwires

An important question concerning the quality of a sensor is how much noise it generates. There are mainly two different positions where noise can arise, in the electrolyte and in the FET. The noise in the bath is generated because of charge transfers across the electrode-electrolyte interface region. For example due to the resistance at the interface (diffuse region) or by redox operations [49]. Further noise arises in the FET, which is generated by thermal or $\frac{1}{f}$ noise on the SiO_2 -channel interface [50]. In this section the noise of the sensor devices will be estimated and the influence of the operating point on the SNR will be analyzed.

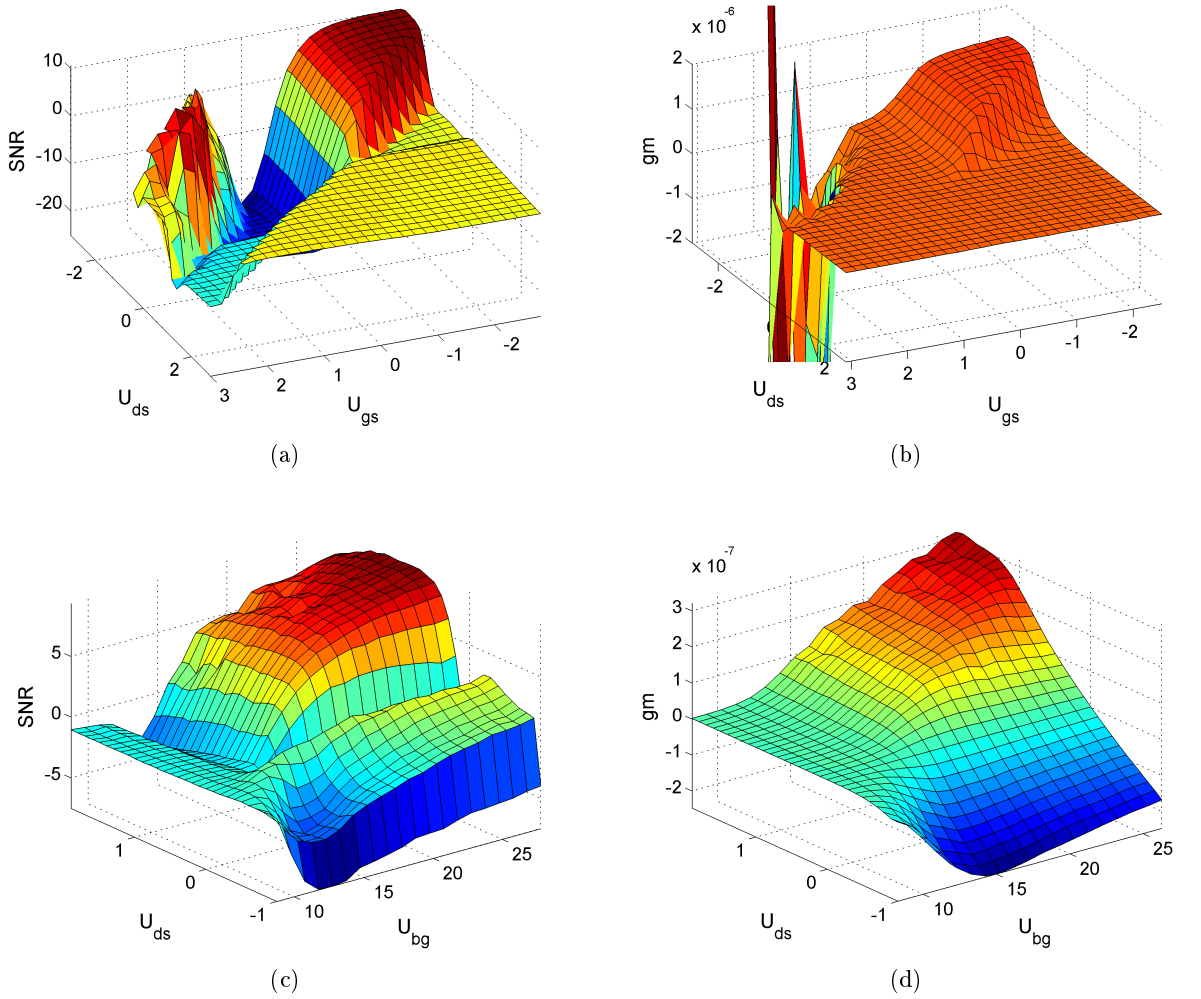


Figure 5.15: SNR (a) and g_m (b) of an ISFET at different operation points. SNR (c) and g_m (d) of a microwire at different operation points.

For an optimum signal detection, it is more important to have a high SNR, than a high signal amplitude. The latter can be adjusted by increasing the gain of the amplifier. In contrast the SNR cannot be improved. Thus, in order to obtain a sensor signal with a high SNR it seems reasonable to set the operating point of the sensor at the maximum of g_m . Though several noise sources are coupled to the transconductance. For example the spectral density of the drain current noise is given by:

$$S_{I_D} = g_m^2 S_{U_G} \quad (5.9)$$

It obviously increases quadratic with the g_m . In contrast the thermal channel noise decreases at the same rate (see [50] for a detailed description of the noise parameters):

$$S_{U_{Si}} = \frac{4kT\mu_{eff}}{g_m^2 L^2} \cdot (-Q_{IT}) \quad (5.10)$$

Since the g_m is a function of the drain and gate voltage, there might be an operating point which does not provide a maximum amplification, but which gives an optimum SNR. Therefore, the SNR and g_m were measured for various operating points and compared to each other. The results can be seen in figure 5.15. The SNR values were determined by the method described in 3.4. Every SNR value was averaged 30 times to have a good approximation of the correct value. At the same time the DC-component was measured. By using equation 5.8 the drain current was calculated and derivated in order to obtain the transconductance. For this measurement the output signal of the SR830 was used as source. It had a SNR of around 22 dB, calculated of the spectral power density between 1 Hz and 100 kHz. The frequency range was important, since the higher the bandwidth the more noise was incorporated in the measurement and thus the lower the SNR became. However, since all measurements were performed with the same settings, they were well comparable.

In figure 5.15(a) and 5.15(b) this measurement was performed for an ISFET. At $U_{ds} \leq 0$ and $U_{gs} \geq 0$ the curve shows some artifacts, because the amplifier saturated at these points. The normal operation point is in the area of $U_{ds} \leq 0$ and $U_{gs} \leq 0$. In this region the SNR values are elevated, with a maximum of 10.6 dB. The same region also showed raised values for the g_m (see figure 5.15(b)). This plateau seems to have a similar shape for the transconductance and the SNR measurements. This suggest that the noise processes are independent of the operating point in this region. By increasing the transconductance only the signal power is elevated which leads to an increase of the SNR. The noise component stays constant at the same time. For $U_{gs} \geq 0$ the SNR strongly decreases.

For comparison this measurement was also repeated for a microwire device. The results are shown in figure 5.15(c) and 5.15(d). In this case the transconductance has a more constant gradient than for the ISFETs. At $U_{bg} \geq 15$ V and $U_{ds} \geq 0$ V the SNR showed a strong increase and only in this region the transconductance was positive. The maximum SNR was 9.3 dB which was very similar to the ISFET devices. In contrast the correlation of the transconductance and

the SNR was not as strong as it was the case for the ISFET devices. The SNR seems to saturate, even if the transconductance further increases. This suggests that there might be additional noise components involved, which limited the SNR for increasing g_m . The SNR seemed to saturate, even if the transconductance further increased. The noise did not seem to become a more severe problem for the microwires than for the ISFETs, since the maximum SNRs were similar.

It can be concluded that it is generally reasonable to set the operating point of the sensor device to the maximum g_m . These measurements show a good agreement between the SNR and the transconductance.

5.6 Microwire modeling

In order to better understand the behavior and to have a better insight for a system, the development of a model is an often used approach. Since the model is used to evaluate the data, it should reproduce the measured results as accurate as possible. Furthermore it should be simple, physically meaningful and allow predictions of the system behavior. In this chapter measured impedance transfer functions of microwires are discussed. At first a short introduction concerning the basics of time constants is given. Thereafter, the measurements are checked for linearity and are described phenomenologically. Finally, this description is used to fit an electrically equivalent circuit (EEC) and to calculate its elements.

5.6.1 Transfer function fundamentals

In the following the sensor is regarded as a circuit of passive resistors and capacitors. Such a circuit can be well described by a transfer function. It is defined by the ratio of the input and the output voltage:

$$H(jw) = \frac{U_{out}}{U_{in}} = |H(jw)| \cdot e^{j\varphi(w)} \quad (5.11)$$

$H(jw)$ is dimensionless and simply describes the attenuation of the system at a specific frequency. As the notation of jw already indicates it is a complex value. As such it can be separated into the magnitude $|H(jw)|$ and its phase component $\varphi(w)$. This is exactly the measured data, which the Lock-In provides.

In system theory [37] such transfer functions $H(jw)$ are often written in a normalized form:

$$H(jw) = \frac{U_{out}}{U_{in}} = V \cdot \frac{(1 + jw\tau_{n1}) \cdot (1 + jw\tau_{n2}) \cdot (1 + jw\tau_{n3}) \cdots}{(1 + jw\tau_{p1}) \cdot (1 + jw\tau_{p2}) \cdot (1 + jw\tau_{p3}) \cdots} \quad (5.12)$$

Every linear system of resistive and capacitive elements can be described by such a function. The big advantage of this representation is that the values can be directly deduced from impedance plots. V is an amplification factor which shifts the magnitude vertically, but has no

influence on the phase. τ_X is a so called time constant. There are two types of time constants: zero (τ_n) and pole (τ_p) points. These determine the frequencies where changes in the magnitude and phase occur.

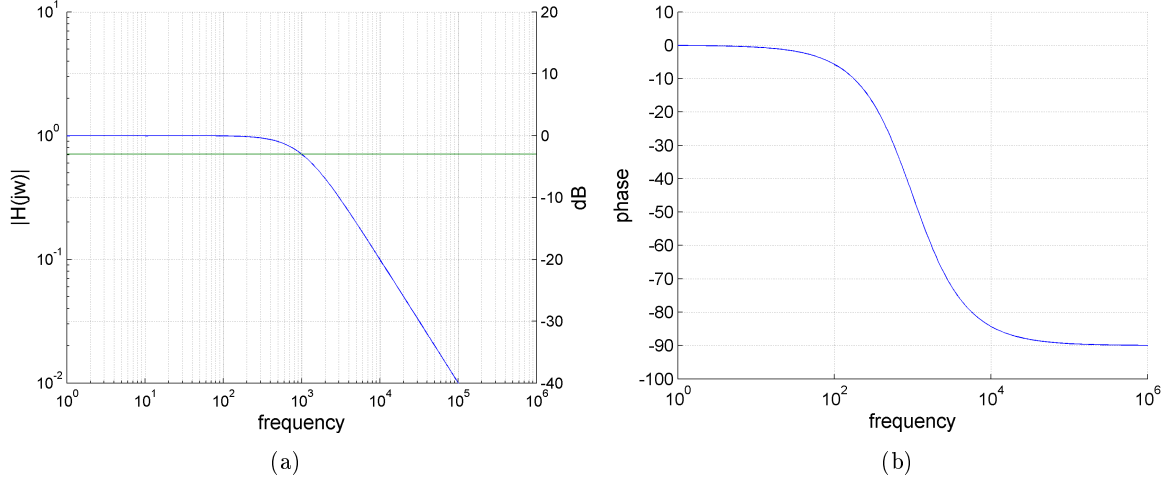


Figure 5.16: **(a)** Magnitude and **(b)** phase of a single pole point at 1 kHz.

As an example the coefficients of equation 5.12 will be deduced from figure 5.16. It shows an example transfer function of a perfectly linear system. To correctly determine the values, the magnitude plot must be logarithmic and the phase plot semi logarithmic. This example only has one time constant. In the magnitude plot it can easily be located at the point when the curve starts to decrease. The exact value of a time constant is defined by the point, when the magnitude has changed by 3 dB from its previous value. In figure 5.16 the green line marks the 3 dB border. Therefore, the time constant is at the frequency $f_g = 1$ kHz. It can be calculated by:

$$\tau = \frac{1}{2\pi \cdot f_g} \quad (5.13)$$

Furthermore, since the magnitude decreases by 20 dB per frequency decade, it is called a pole point τ_p . If it would increase by the same slope it would be a zero point τ_n . Every time constant has an influence on the magnitude and also on the phase. In the case of a pole point the phase decreases by 90° , beginning and ending one decade before and after the pole point. If it is a zero point the phase would increase accordingly. Time constants can also be negative. This has no influence on the magnitude, but the phase slope is inverted. Thus a negative pole point would have an increasing phase. Time constants can also have a higher order as shown in the following equation of a pole point of order n :

$$H_n = \frac{1}{(1 + jw\tau_{p1})^n} \quad (5.14)$$

In principal they behave the same as time constants of the first order, but their slope is increased. For example at a time constant of second order the slope increases by 40 dB/dec and there is an overall change of 180° in the phase. The influence of time constants on transfer functions is summarized in table 5.1.

		Magnitude		Phase	
	Prefix	1 st order	n^{th} order	1 st order	n^{th} order
Zero-point	+	↗ by 20 dB/dec	↗ by $n \cdot 20$ dB/dec	↗ by 90°	↗ by $n \cdot 90^\circ$
	-			↘ by 90°	↘ by $n \cdot 90^\circ$
Pole-point	+	↘ by 20 dB/dec	↘ by $n \cdot 20$ dB/dec	↘ by 90°	↘ by $n \cdot 90^\circ$
	-			↗ by 90°	↗ by $n \cdot 90^\circ$

Table 5.1: Influence of time constants on the transfer functions.

Besides the time constants also the factor V can be identified. This is done by considering equation 5.12 at low frequencies:

$$\lim_{w \rightarrow 0} H(jw) = V \cdot \frac{(1 + jw\tau_{n1}) \cdots}{(1 + jw\tau_{p1}) \cdots} = V \quad (5.15)$$

In our example this gives $H(jw \rightarrow 0) = V = 1$. The overall transfer function of figure 5.16 is:

$$H(jw) = \frac{1}{(1 + jw\tau_{p1})} \quad \tau_{p1} = \frac{1}{2\pi \cdot 1 \text{ kHz}} \quad (5.16)$$

5.6.2 Phenomenological microwire description

In order to analyze and explain the microwire behavior it would be interesting to apply the just presented method to the measured transfer functions. In a first step the influence of the electronics must be subtracted from the curves. This is important since only the sensor behavior is of interest. As was shown in subsection 5.4.3, the amplifier always has a remaining influence, which should be compensated. Therefore only the transfer function of the amplifier was measured as a reference (see subsection 5.4). Thereafter it was subtracted from the actual sensor measurements. As was already mentioned, this correction was not ideal and could only reduce the error of the electronics. Especially at high frequencies the transfer functions should not be regarded as being exact.

In figure 5.17 an example of a measured transfer function of a microwire in an electrolyte of 0.1mM NaCl concentration is shown:

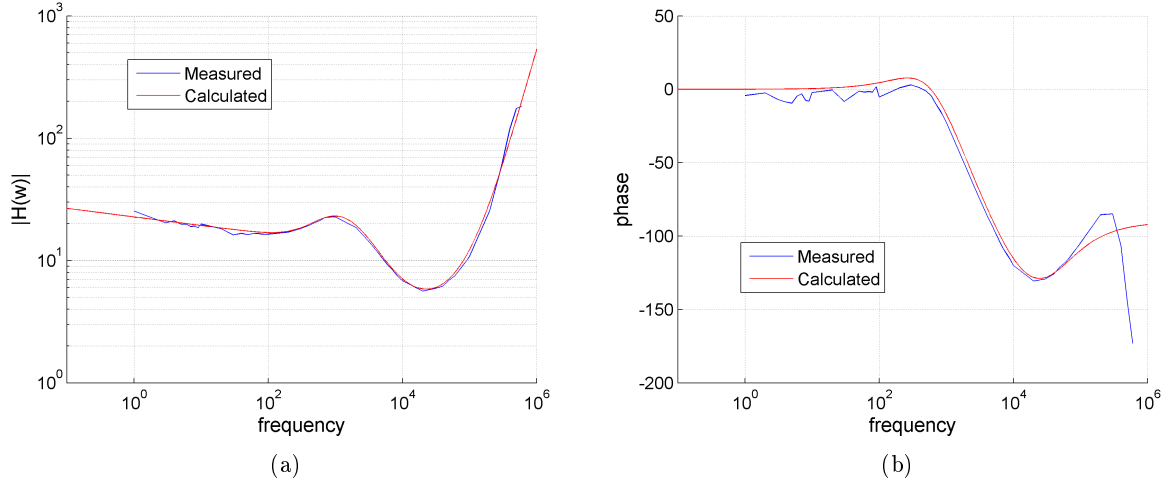


Figure 5.17: **(a)** Magnitude and **(b)** phase of a microwire immersed in 0.1mM NaCl.

The measurement was performed by the self developed Lock-In. Based on the method presented in the previous subsection, time constants were fitted to the curve (see equation 5.12). At 0.1mM NaCl all time constants can be easily distinguished. To describe the curve three zero and two pole points are necessary. The first zero point is around 350 Hz and leads to an increase in magnitude. At 750 Hz and 1.8 kHz two pole points follow. The first one compensates the previous zero point. If there were no other time constants the magnitude would remain flat from this point. However the second pole point decreases the magnitude. At 8.6 kHz and 48.6 kHz two zero points act the same way. In contrast to the pole points they lead to an increase of the magnitude. In theory the time constants could also be deduced from the phase information. However the overlap of the phase changes makes it difficult to distinguish the time constants. Nevertheless the phase is well suited to improve the accuracy of the chosen frequencies, since changes in the time constants have an higher impact on the phase than on the magnitude. Additionally, the phase determines the sign of the time constants. Thus the second zero point at 8.6 Hz is chosen to be negative. If would be chosen to be positive there would be no good agreement of the magnitude and phase at the same time. At high frequencies the magnitude and phase do not fit to the calculation well any more. It is unclear if this is a compensation problem or if there are additional time constants at the border of the measurement range. Both possibilities cannot be fully verified. Thus this discrepancy will not be regarded in this work.

In figure 5.18 the same measurement as in figure 5.17, but for several different NaCl concentrations is shown. At first glance, the curves seem to have very varying shapes. To elaborate the differences, it is favorable to observe the changes of the time constants. Therefore the process to determine the time constants was repeated for all curves. The results are summarized in table 5.2. In figure 5.18(a) at the transition between 0.01 mM and 0.1 mM NaCl, the time constants τ_{n1} and τ_{p2} seem to disappear. Below 0.1 mM NaCl a good but not perfect fit can be attained by only using three time constants. However, it seems improbable that two time constants just

disappear. At high NaCl concentrations above 0.1 mM τ_{p2} increases approximately linearly. In contrast τ_{n1} stays nearly constant. This suggests that at 0.01 mM τ_{n1} and τ_{p2} nearly have the same value. Therefore they compensate each other (see equation 5.12) and cannot be deduced from the graphs. However it stays unclear why τ_{p2} does not reappear at low NaCl concentrations. It could be expected to further decrease. Thus it was also tried to find values for τ_{n1} and τ_{p2} below 0.1 mM NaCl. Since it can be assumed that τ_{n1} stays between 300 Hz and 400 Hz and τ_{p2} was chosen accordingly. In table 5.2 these values are marked in a light gray. This problem will be further addressed in subsection 5.6.5, when the time constants are interpreted. At 10 mM NaCl τ_{n3} disappeared. In this case it simply leaves the measurement range.

With this first abstraction of the measurement data, a phenomenological description was deduced of the transfer functions. It can be observed that τ_{n3} and τ_{p2} seem to be proportional to the NaCl concentration. τ_{n1} stays constant, while τ_{n2} and τ_{p1} slightly increase. Additionally it was verified that the measurements can be described by normalized transfer functions like equation 5.12. This sustains the assumption that the measured system behaves linear. In order to model the transfer curves by a network of passive linear elements this is an important prerequisite. However, in figure 5.18(a) a small decrease of the magnitude can be observed at low frequencies (1-100 Hz) with increasing NaCl concentrations. In this region the phase remains constant. Such a behavior cannot be modeled by time constants. Thus $|H(jw)|$ needs to be multiplied by a correction factor $w^{-0.07}$ to take this effect into account. In the next chapter this factor will be further discussed. Finally, the measured curves are fully described by the values in table 5.2 and the normalized transfer function is given by:

$$H(jw) = V \cdot w^{-0.07} \cdot \frac{(1 + jw\tau_{n1}) \cdot (1 + jw\tau_{n2}) \cdot (1 + jw\tau_{n3})}{(1 + jw\tau_{p1}) \cdot (1 + jw\tau_{p2})} \quad (5.17)$$

5.6.3 Electrically equivalent circuit (EEC)

In this section it was tried to find an interpretation of the time constants deduced in the previous sections. To do this an EEC is presented which relates the time constants to physically meaningful elements. It is tried to keep the overall system as simple as possible and to find an easy mathematical description. By calculating its time constants the elements of the EEC can be determined. This finally offers the possibility to interpret the measured data. Finally, limits and problems of the EEC will be discussed.

To be able to fit the elements of an EEC to the measured curves, its transfer function must resemble equation 5.17. Thus it must have at least five different time constants. Furthermore it must be proportional to the correction factor $w^{-0.07}$. An EEC which fulfills these requirements is shown in figure 5.20.

Since this is a small signal EEC the supply voltages are not considered. At the right bottom the sensor itself is modeled by the equivalent circuit of a FET. This is appropriate since the microwires have FET-like behavior (see chapter 2.1.2). Thus C_{Ox} is the gate capacitance. g_m is the transconductance of the FET. Multiplying g_m by the gate voltage u_g gives the drain source

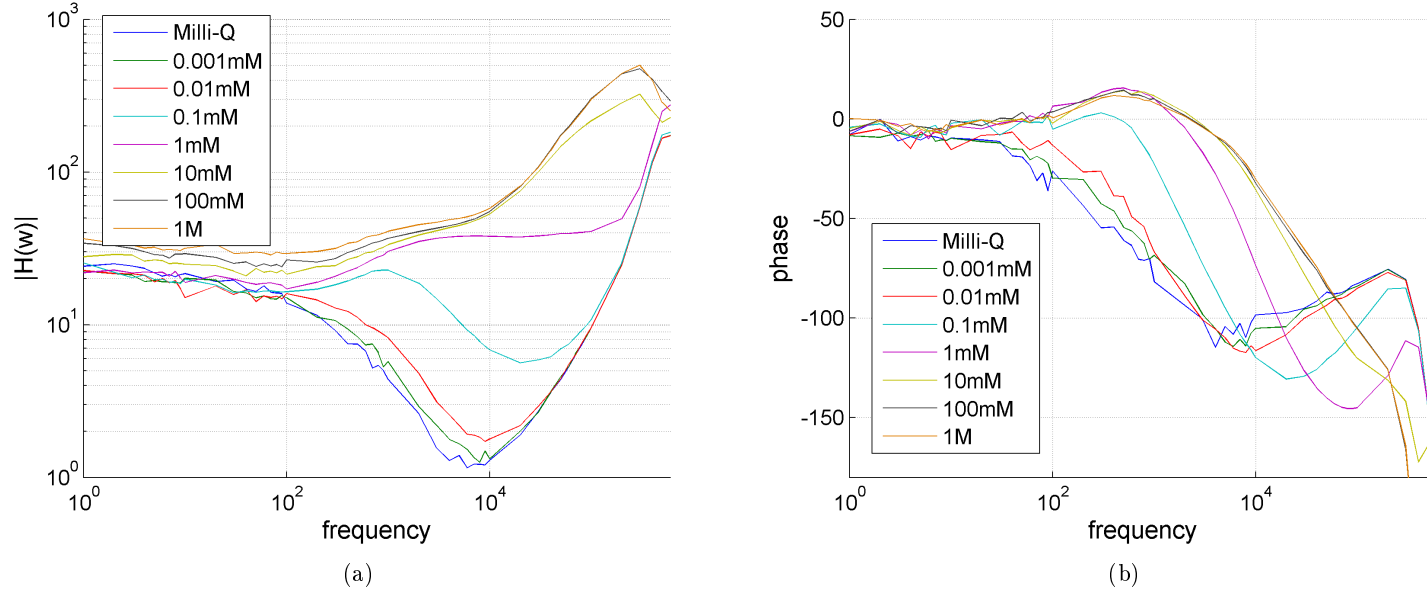


Figure 5.18: Measured **(a)** magnitude and **(b)** phase of a microwire in solution for different NaCl concentrations.

NaCl:		Milli-Q	0.001mM	0.01mM	0.1mM	1mM	10mM	100mM	1M
Zero-points	τ_{n1}	400 Hz	350 Hz	350 Hz	354 Hz	400 Hz	307 Hz	409 Hz	322 Hz
	τ_{n2}	-3.2 kHz	-4.6 kHz	-4.5 kHz	-8.6 kHz	-10.9 kHz	-8.8 kHz	-9.2 kHz	-10.7 kHz
	τ_{n3}	8.8 kHz	10.7 kHz	16.4 kHz	48.6 kHz	409.8 kHz			
Pole-points	τ_{p1}	550 Hz	550 Hz	661.2 Hz	757 Hz	990 Hz	728 Hz	882 Hz	661 Hz
	τ_{p2}	220 Hz	250 Hz	370 Hz	1.8 kHz	13.6 kHz	72.7 kHz	117.4 kHz	135.5 kHz
Factor	V	27.5	25	25	25	27.5	33	38	42

Table 5.2: The time constants deduced from figure 5.18. For clearness they are given as cut-off frequencies in hertz.

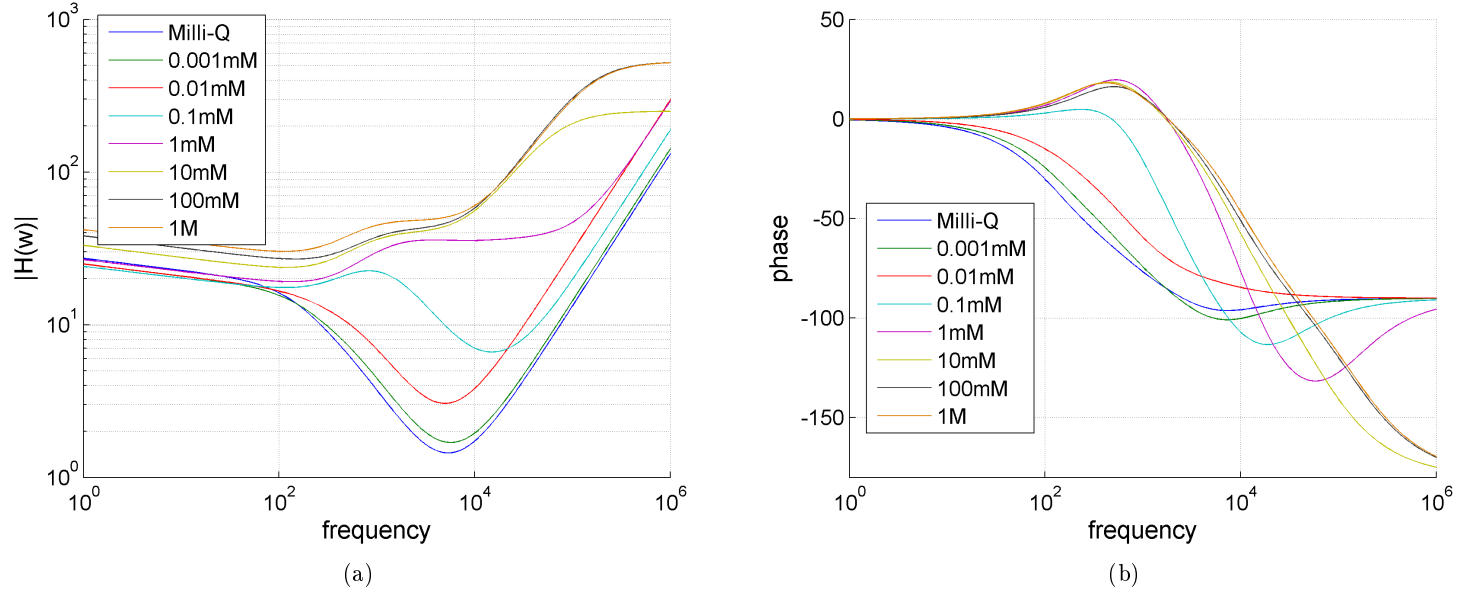


Figure 5.19: (a) Magnitude and (b) phase of a sensor in NaCl calculated by an EEC.

NaCl:	Milli-Q	0.001mM	0.01mM	0.1mM	1mM	10mM	100mM	1M
R_{EL}	11.5 $M\Omega$	9.1 $M\Omega$	6.3 $M\Omega$	1.9 $M\Omega$	0.2 $M\Omega$	40 $k\Omega$	19 $k\Omega$	19 $k\Omega$
R_M	0.6 $G\Omega$	1.0 $G\Omega$	1.3 $G\Omega$	1.5 $G\Omega$	1.5 $G\Omega$	1.6 $G\Omega$	1.2 $G\Omega$	1.6 $G\Omega$
C_M	0.62 pF	0.46 pF	0.36 pF	0.31 pF	0.26 pF	0.29 pF	0.31 pF	0.31 pF
g_m	10.7 μS	9.2 μS	9.15 μS	12.5 μS	12.8 μS	11.7 μS	13.4 μS	15.4 μS
C_G	2.19 pF	2.36 pF	5.24 pF	3.27 pF	5.23 pF			
C_{DL}	23 pF	24.5 pF	24.7 pF	17.6 pF	19 pF	25.4 pF	25.7 pF	24.5 pF
δ_{NaCl} [Ω cm]	$1.33 \cdot 10^6$	$4.72 \cdot 10^5$	$3.6 \cdot 10^5$	$7.89 \cdot 10^4$	$9 \cdot 10^3$	$9.4 \cdot 10^2$	$1 \cdot 10^2$	$1.26 \cdot 10^1$

Table 5.3: Calculated values for the elements of the EEC, based on the values in table 5.2 ($C_{Ox} \approx 0.17$ pF and $C_L \approx 0.5$ nF).

C_M and R_M model the Helmholtz layer which is built up at the interface between SiO_2 and electrolyte (for further information see [10]). R_{EL} is the resistance of the electrolyte. It depends on the distance of the sensor to the electrode and on the conductivity of the electrolyte. C_G is a parasitic capacitance, which is due to the geometric arrangement of the setup. The surface of the electrode and chip can be thought of as a plate capacitor. Thus the capacitance can be estimated by:

$$C_G \approx \epsilon_0 \cdot \epsilon_{r,\text{H}_2\text{O}} \cdot \frac{A}{d} \approx 0.7 \text{ pF} \quad \epsilon_{r,\text{H}_2\text{O}} = 80 \quad A = \pi \cdot 0.00125^2 \text{ m}^2 \quad d = 0.005 \text{ m} \quad (5.19)$$

A is the size of the electrode surface (it has a diameter of 2.5 mm specified by the manufacturer) and d is the distance between the chip and the electrode. C_G is neither a value of interest, nor is it easy to measure and as such to verify. Several attempts to find an alternative for the EEC without this capacitance showed its importance to get a good fit to the measured data. Furthermore, due to the high dielectric properties of water [51] it is a quite large capacitance. Later on its value will be calculated from the time constants, showing a good agreement with equation 5.19. In addition it is in parallel to the large electrolyte resistance R_{EL} and as such it easily generates a time constant in the measurement range.

As a last compartment C_{DL} models the double layer at the reference electrode. This is not a usual capacitance, but a so called constant phase element (CPE) [29]. Its impedance can be calculated by:

$$Z_{DL} = \frac{1}{j(w \cdot C_{DL})^n} \quad (5.20)$$

In literature n has a value around 0.97 (see [52]). This effect is probably due to the surface roughness of the Ag/AgCl electrodes. There exists no simple mathematical description of n , but there are numerical approaches to calculate it. It can be used to describe the small decrease in magnitude at low frequencies (see figure 5.18(a)) as was mentioned in the last chapter.

5.6.4 Analytical solution of the EEC

The next step is to calculate the elements of the EEC, based on the measured data. Even though the model was simplified as much as possible, it consists of numerous elements. This makes it hard to perform a numerical fit of the elements. There are many degrees of freedom which will make it very improbable, that such an approach will converge. Therefore it is favorable to calculate the normalized transfer function of the EEC. As mentioned in the last chapter, it corresponds very well to the phenomenological description for the given EEC (equation 5.17). The time constants can be equated to the values in table 5.2. This allows to exactly calculate the elements of the EEC for a given set of time constants. The detailed calculation of the EECs transfer function are shown in appendix C. The resulting function is:

$$H(jw) = R_A \cdot g_m \cdot w^{n-1} \cdot \frac{C_{DL}^n}{2C_L} \cdot \frac{(1 + jw\tau_{n1}) \cdot (1 + jw\tau_{n2}) \cdot (1 + jw\tau_{n3})}{(1 + jw\tau_{p1}) \cdot (1 + jw\tau_{p2})} \quad (5.21)$$

$$\tau_{n1} = C_M \cdot R_M \quad \tau_{n2} = -C_L \cdot \frac{C_M + C_{Ox}}{g_m \cdot C_M} \quad \tau_{n3} = R_{EL} \cdot C_G \quad \tau_{p1} = R_M \cdot (C_M + C_{Ox}) \quad (5.22)$$

Equation 5.21 shows a good agreement with the phenomenological transfer function. Comparing equations 5.21 and 5.17 the following informations can be deduced:

- Using the relation $w^{-0.07} \stackrel{!}{=} w^{n-1}$, the value of the CPE element n can be calculated to 0.93. This value is well consistent with data published in literature [52].
- In $V = R_A \cdot g_m \cdot \frac{C_{DL}^n}{2C_L}$, R_A is the resistance of the impedance converter. It appears that V is directly proportional to g_m . This results in the rather trivial conclusion that the sensor signal can be increased by a higher transconductance g_m .
- In table 5.2, τ_{n1} remains constant for all NaCl concentrations. This suits the analytical solution very well, since neither R_M nor C_M should depend on the NaCl concentration of the electrolyte.
- τ_{n2} is always negative. This holds true for the calculated solution and for the values deduced from the graphs.
- τ_{n3} is proportional to the resistance R_{EL} . This explains the fast change of the time constant in table 5.2.

These analogies of the model and the measured data show that the model is well applicable to the microwire devices. However there are also some problems and discrepancies.

- First of all equation 5.21 and 5.22 are only an approximation. It was necessary to make some simplifications, since an easy and exact solution could not been found due to the complexity of the EEC. In general the error of the approximation is neglectable. This holds true as long as the elements fulfill the following prerequisite:

$$\begin{array}{ccccccccc} R_M/\Omega & \gg & R_{EL}/\Omega & \gg & g_m/S & \gg & C_L/F & \gg & (C_{Ox}, C_M, C_G, C_{DL})/F \\ 10^{13} & & 10^6 - 10^3 & & 10^{-5} & & 10^{-10} & & 10^{-12} - 10^{-13} \end{array} \quad (5.23)$$

- It was not possible to find an analytical solution for τ_{p2} . The reasons are discussed in greater detail in the appendix C. However, a deeper understanding of this time constant would be desirable, since it apparently was also proportional to R_{EL} .
- Since resistors and capacitors are always positive, τ_{p1} should always be greater than τ_{n1} . In table 5.2 the contrary is the case (the values have to be converted from hertz to seconds). When the values of the elements are calculated, some become negative. This is very unsatisfactory since the only explanation is that the model does not fit to the applied data. However, there could not be found a solution to this problem. Thus it requires further investigation in future works. In this thesis only the absolute values of all calculated elements are shown. Despite their prefix, these values seem to have reasonable values.

5.6.5 Calculation of EEC elements

By using equation 5.21, 5.22 and the values in table 5.2, the elements of the EEC can be calculated. In the system of equations there are eight unknown (R_{EL} , C_M , ...) and six known variables (V , τ_{n1} , ...). To find a solution, two additional values have to be provided. C_{Ox} and C_L were chosen since these always stayed constant and could be calculated from the layout information. For a typical microwire they were approximately:

$$C_{Ox} \approx 0.17 \text{ pF} \quad C_L \approx 0.5 \text{ nF}$$

Thus every combination of V , τ_{n1-3} and τ_{p1} provides exactly one solution for R_M , C_M , C_{DL} , g_m and C_G . R_{EL} finally must be chosen manually since there is no analytical solution for τ_{p2} . In table 5.3 the results of the calculations are summarized and in figure 5.19 the transfer functions based on the calculations are shown.

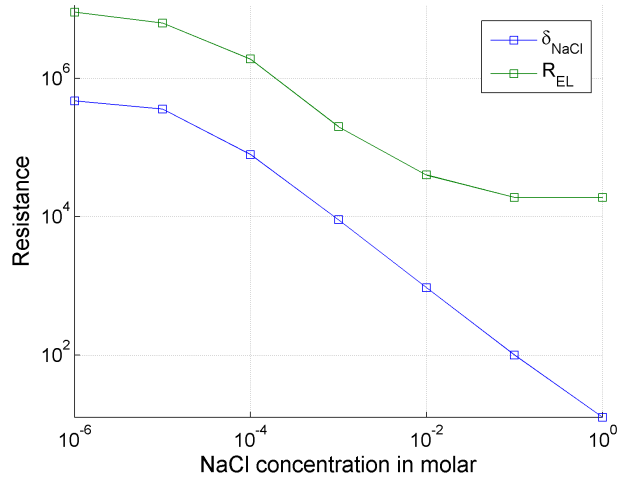


Figure 5.21: Comparison of measured δ_{NaCl} and calculated R_{EL} .

C_G could not be calculated for NaCl concentrations above 1 mM, because τ_{n3} left the measurement range. Furthermore, the values below 0.1mM must be regarded carefully. In this range τ_{n1} and τ_{p2} could not be located exactly as was already described in subsection 5.6.2.

As expected, most elements do not show a big change and are in a physically meaningful range. An exception is R_{EL} . Since it is linearly dependent on the NaCl concentration in the electrolyte it varies over four decades. At the bottom of table 5.3, the conductivity δ_{NaCl} of the electrolyte was also measured by an external device. Theoretically both values R_{EL} and δ_{NaCl} should be proportional to each other. In [50] the relation of both values is given by:

$$R_{EL} \approx \delta_{NaCl} \cdot \sqrt{\frac{\pi}{W \cdot L}} \quad (5.24)$$

W and L are the dimensions of the gate. In figure 5.21 R_{EL} and δ_{NaCl} are plotted against the NaCl concentration. Both curves show a good agreement until 10 mM of NaCl. The discrepancy at higher concentrations probably arises, since τ_{p1} and τ_{n3} reach and cross the border of the measurement range. Figure 5.21 also explains why τ_{n1} and τ_{p2} seem to disappear below 0.1 mM NaCl. At this point the conductivity does not increase linearly anymore with decreasing concentrations. This is probably due to a impurity of the Milli-Q water, which limits the conductivity to a certain value. Thus the time constant τ_{p2} effectively stops to decrease to lower values and does not reappear.

Several measurements of different NaCl concentrations showed a vertical shift in the magnitude for higher concentrations. This shift can only be induced by changes in C_{DL} or g_m . The other variables in the factor V remain constant. Table 5.2 shows that the value of g_m changes by 50%. A possible explanation might be the potential of the reference electrode. By the big increase of NaCl in the electrolyte its potential can change by several folds of 10 mV. This is enough to shift the operating point and as such, alter the g_m . Another explanation could be a leakage current in the gate oxide which increases, when the conductivity of the electrolyte is improved.

5.7 Biological measurements

Apart from using sensors to detect molecules, they can also be used to measure cell behavior, e.g. action potentials or cell adhesion (see section 2.2 for further details). In the following cell adhesion and cell death will be analyzed by measurements with ISFET devices. For these measurements the Lock-In developed in this thesis was used.

In a first step it will be shown that cell adhesion can be in principle recorded by impedance measurements. By repeatedly measuring, time resolved changes in the cell adhesion will be monitored. Finally a controlled cell death will be observed by ISFET measurements.

5.7.1 Mechanical cell removal

In order to show that cells have an influence on an ISFET, the transfer functions were measured before and after a cell on top of the gate was removed. An example of a gate covered by several cells is shown in figure 5.22(a) and the corresponding normalized transfer functions of this chip are shown in figure 5.22(c). Broken channels and gates with similar transfer functions are not shown.

A general problem for cell measurements is that the cell position on the chip cannot be influenced directly. Neither the overall location of the cell on the chip, nor the exact adhesion on the gate can be dictated. Thus a gate can be covered by a single or multiple cells, by the entire cell body or just by an extension. Even identically looking cells can be adhered differently. All these variations leave space for differences of the transfer functions. Thus, even though there were cells

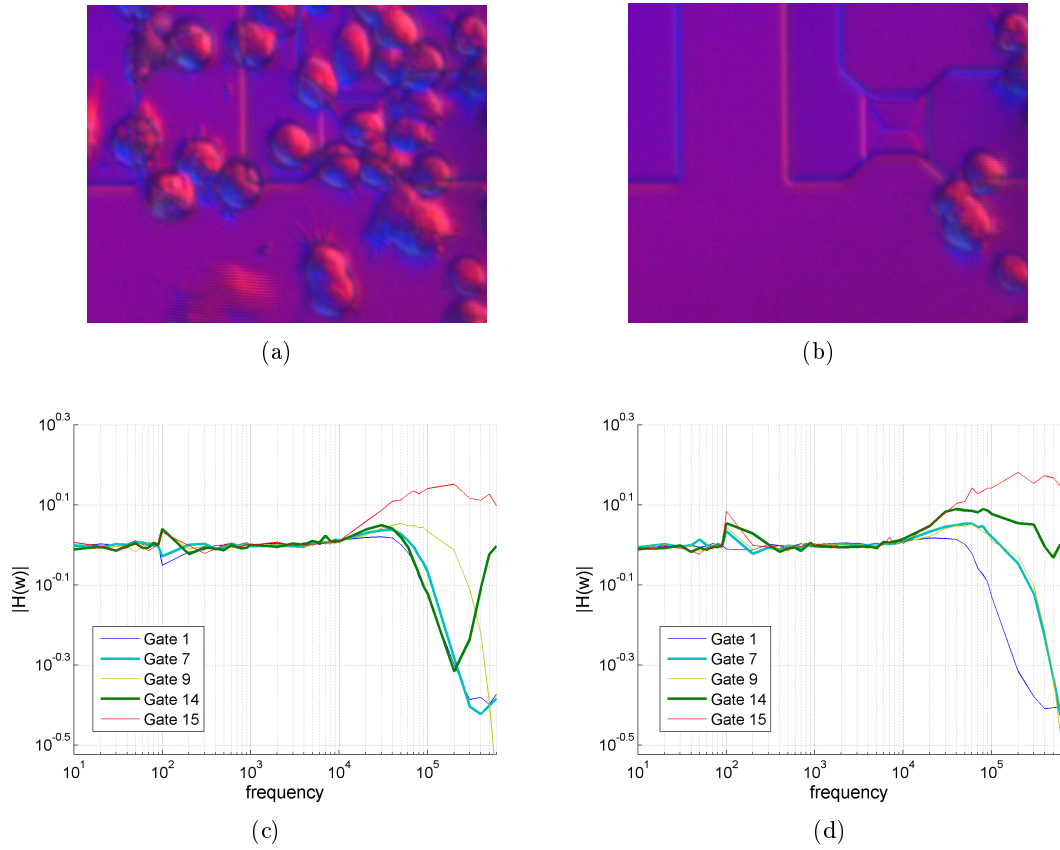


Figure 5.22: **(a)** Image of gate 14 of an ISFET covered with cells. **(b)** The same gate, after the cells were removed mechanically. Magnitude of the transfer function before **(c)** and after **(d)** cell removal.

on gate 1, 7 and 14 (the cells of gate 14 are shown in figure 5.22(a)), the transfer functions have partly different shapes (see [16] for a further discussion). Optical observations suggest, that cells which adhere very good have a more distinct minima. Thus the cells on gate 1 and 7 are better adhered than on 14. Even though the gates 9 and 15 are empty they show differences in their transfer function. This might be due to differences of the ISFETs or due to the protein coating, which covers the entire chip.

To show that the transfer functions are affected by the cell, they were removed on gate 7 and 14 by the tip of a pipette. The result is shown in figure 5.22(b) and 5.22(d). Obviously the shape of the transfer functions of both gates changes. They now resemble to the empty gates 9 and 15. Gate 1 was unaffected of this change, since there the cell was not removed. There is also a small, noticeable overall change in the transfer function. This is probably because the reference electrode was slightly moved during the experiment.

5.7.2 Measured trypsin activity

To further investigate the influence of the cell adhesion on the transfer functions, trypsin measurements were performed. By adding trypsin the connections between the cell and the protein layer are cleaved. This weakens the cell adhesion, which can also be observed optically. In figure 5.23(a) and 5.23(b) a cell on a gate is shown, before and after trypsin was added. When the cells had a good adherence they were spanned on the chip surface by focal adhesions points. These adhesion points seem to pull the cell on the chip as can be seen in figure 5.23(a). During trypsin activity these connections are cleaved which leads to a change in morphology towards roundly shaped cells (see figure 5.23(b)).

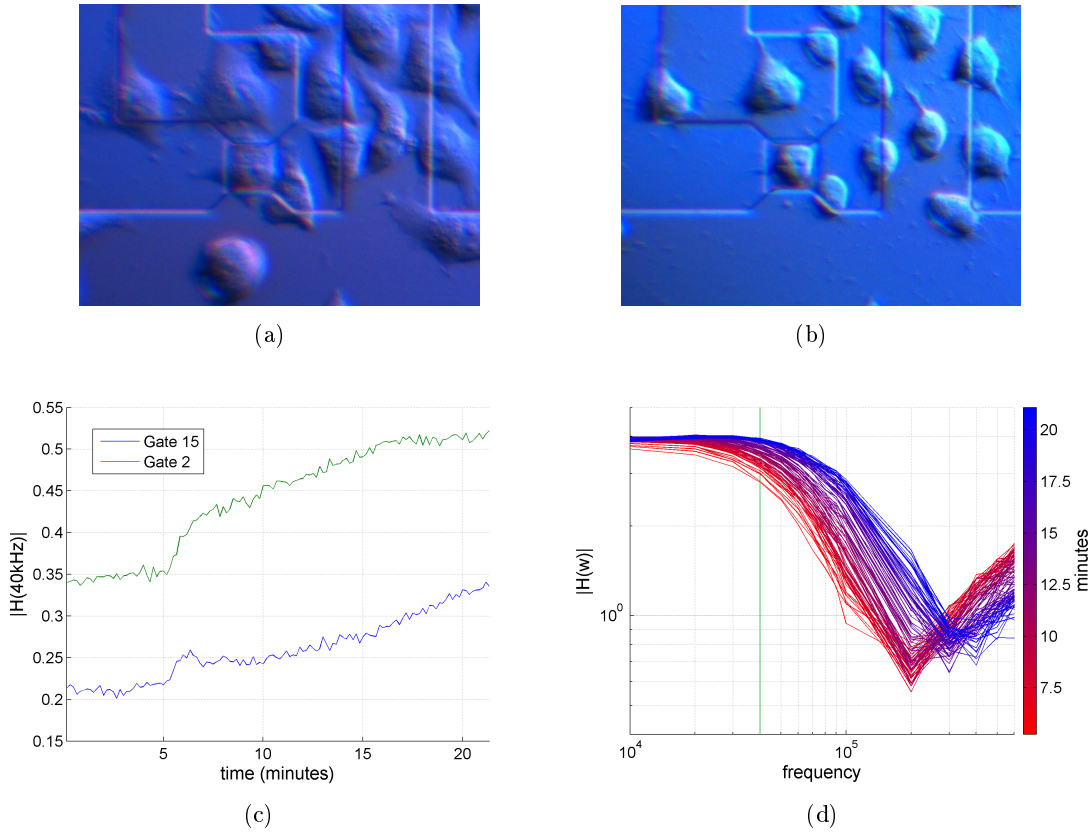


Figure 5.23: Adhered cell on gate two before **(a)** and after **(b)** trypsin activity. Change of the transfer function for a single **(c)** and multiple frequencies **(d)** during trypsin activity.

To monitor the changes of the cell adhesion, transfer functions were measured repeatedly. The trypsin induced changes of the transfer function can happen at a fast rate in the range of minutes. To correctly record these change the measurement was optimized to achieve a high repetition rate. Therefore only a few gates were measured in a limited frequency range (see also section 4.1).

In figure 5.23(c) the changes of the transfer function at 40 kHz are shown for two gates. To ensure all changes are due to the trypsin activity, the first five minutes the cells were only immersed in PBS. During this period there were no relevant changes in the transfer functions. This confirmed that the system was stable and other influences can be neglected. Then, after 5 minutes the trypsin was added. Immediately all transfer functions showed a large increase which can be explained by the changed conductivity of the electrolyte. It is well known that the electrolyte resistance has an important influence on the transfer function. Thereafter a slow increase can be observed on several gates. As figure 5.23(c) indicates this process does not start simultaneously on all gates. Gate two immediately increases, while gate 15 seems to be delayed for several minutes. On other gates no differences can be observed. An explanation could be the different positions of the cells on the gates. As already mentioned there is always a strong variation on the cell location and adhesion.

To get a better insight the transfer functions at different times are shown in figure 5.23(d). In this plot only the curves after the trypsin was added ($t \geq 5$ min) are shown. The color bar indicates in minutes at what time the curves were measured. Obviously an overall shift of the transfer function to higher frequencies can be observed. Thus the increase in figure 5.23(c) can be easily explained. Furthermore it appears that the shift does not slow down. It is expected that the curves will continue to change until they reach a shape, comparable to the case where no cell is adhered to the gate.

5.7.3 Cell apoptosis detection

The term apoptosis describes the process of a programmed cell death. During apoptosis the components of the cell are decomposed in a controlled way. In a living organism this prevents damage caused by remaining parts of the cell. Under the microscope it can be observed, that the apoptosis process leads to cell shrinkage, loss of adherence and an effect called blebbing. The cytoskeleton of the cell is metabolized which leads to irregular bulges on the membrane, called blebs. At a late stage of apoptosis the cell breaks up and the remaining components are encapsulated in vesicles. A more detailed description of apoptosis can be found in [53].

The underlying pathway which induces the apoptosis is quite complex and still not fully resolved. As one step in the apoptosis process a change in the intracellular pH (pH_i) was observed [54]. It generally shows an acidification, typically between 0.3-0.4 pH_i [55]. As already mentioned the pH can be observed by ISFETs. During the apoptosis, the cell adherence decreases. Furthermore the cell membrane becomes permeable and as such, should acidify the surrounding of the cell. These changes, of the adherence, the pH and the permeability should be measurable by the ISFET devices. Therefore in the following measurements the changes of the DC and AC component will be monitored for cells. Since kidney cancer cells were used, the anti-cancer agent CPT-11 was used to induce apoptosis.

Prior to the apoptosis measurements, several analysis were made to allow a more detailed interpretation of the curves. Therefore the sensors pH-sensitivity was calibrated, to allow an

interpretation of the change in the DC component. Furthermore a measurement without CPT-11 was performed to analyze how constant the system behaves. This was done to ensure that the measured effects can be assigned to the apoptosis. Thereafter the actual apoptosis measurements are shown. All measurements in the following subsections are performed with the same electronics and software to exclude additional systematic errors.

pH calibration of sensor

Figure 5.24 shows the voltage change when solutions of different pH are measured. The three curves belong to different ISFETs. On the ordinate the equivalent gate voltage v_{gs} , which would correspond to the voltage applied on the gate, is shown. It is calculated from the output voltage v_o of the amplifier as:

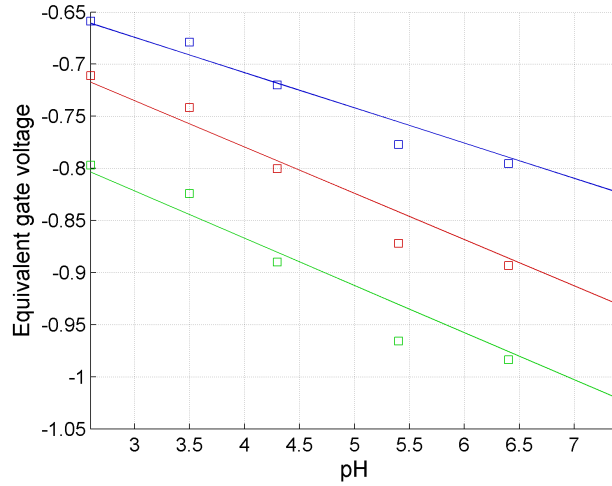


Figure 5.24: pH measurement of several ISFETs. The lines are fitted to the measured data, which are marked by squares. On the ordinate the calculated equivalent gate voltage is shown.

$$v_{gs} = v_o \cdot \frac{1}{R_A \cdot g_m} \quad \text{with} \quad R_A = 5 \, k\Omega \quad g_m \approx 0.1 \, mS \quad (5.25)$$

R_A is the gain of the amplifier and g_m the transconductance of the ISFET. The squares mark the actual measured points. It is expected that the curves show a linear decrease, when the pH of the solution is raised. Therefore the measured points of each ISFET are fitted to a line, which is also shown in figure 5.24.

Obviously the measured points show a good agreement with the fitted line. However there seems to be a systematic variance, e.g. at a pH of 5.5 all points are below the fitted line. Probably this is due to a small measurement error by the pH-meter, since the error seems similar on all ISFETs. Nevertheless the overall slope was around 30.6 mV/pH which is in good agreement

with literature for SiO₂-electrolyte interfaces. [28, 12]

Static system behavior

In order to distinguish effects which are due to the CPT-11 from other influences, the measurement was performed without the anti-cancer agent. Besides the absence of the CPT-11 everything was done like in the apoptosis measurement in the next subsection (the composition of the used buffer can be found in D).

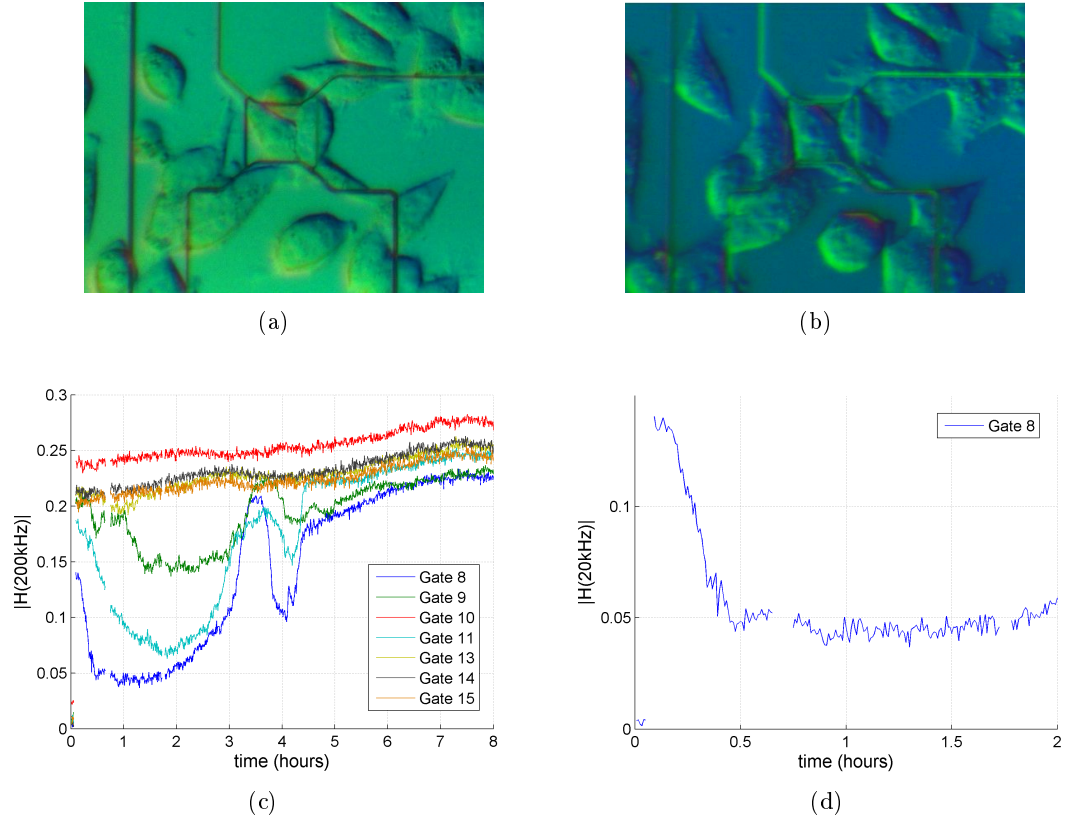


Figure 5.25: Picture of cells on gate eight at the beginning (a) of the measurement and after 40 minutes (b). Corresponding AC component of several (c) and a single (d) gate.

In figure 5.25(c) the transfer functions at 200 kHz are shown as a function of the measurement time. There were cells on the gates 8, 11 and 9 which showed a relatively large change in the AC part. In contrast the gates 10, 13, 14 and 15 were not or only partly covered by cells. They remain more constant than the previously mentioned gates. Probably the changes are due variations of the cells adherence on the gates. In order to better distinguish the changes in the transfer function of gate eight, it is shown for a shorter time period in figure 5.25(d).

In this figure, there are three small gaps at 0.1, 0.7 and 1.7 hours. At these times pictures of the cells were taken by a microscope. There was no data measured during this time, because the additional photocurrents due to the illumination would disturb the measurements. In figure 5.25(a) the corresponding pictures of the cells on gate eight at $t = 0$ and in figure 5.25(b) at $t = 40$ min, are shown respectively. During these first 40 minutes, the transfer function showed a big change at 200 kHz. Compared to the observation of the trypsin measurement, the figures suggest that the cell adhesion has improved. The morphology changes and the cells seem to use more surface to achieve a good adherence. Thus the large fluctuations in figure 5.25(c) are due to the changing cell adhesion. This is feasible since the cell constantly rebuilds its adhesion points. Since the cells are not in a culture medium, their activity decreases after four hours.

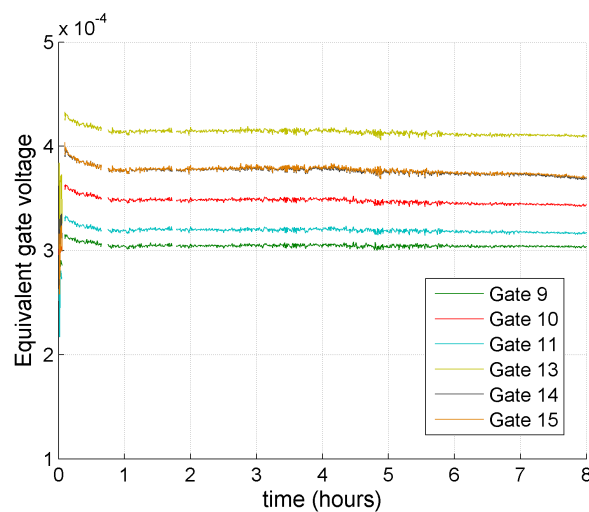


Figure 5.26: Constant component of several gates during measurement without CPT-11.

At the same time the constant DC component of the ISFETs was measured. The result is shown in figure 5.26. An ISFET which was not covered by a cell was chosen as reference gate. Its values were subtracted from all other measured DC components. Obviously the DC component was much more stable and showed no variations. Thus it was independent from changes in the cell adhesion.

Cell apoptosis

The measurement from the previous subsection was repeated this time with CPT-11 added to the buffer solution. In figure 5.27(a) the impedimetric and in figure 5.27(b) the potentiometric measurement of gate 12 are shown. At $t = 0$ the culture medium was exchanged by the mixture of fluorescence markers and CPT-11. Thereafter a big change can be observed in the transfer function. The previous measurements suggest that this is due to a better adhesion of the cell

on the chip. Besides the electrical measurements, fluorescence pictures were taken at regular intervals as it was described in subsection 4.2.2. In figure 5.28(a) a picture of the cell on gate 12 is shown after 1 hour 30 minutes. There is not yet a fluorescence, thus the apoptosis did not take place yet. However, half an hour later the red fluorescence in figure 5.28(b) was very distinct. There is also a green fluorescence from the annexin-V, but it is much weaker and covered by the strong signal of the propidium iodide. During this period a significant change can be seen in the impedimetric measurement. The transfer function was shifted to higher frequencies which suggests a weaker adhesion. There were also changes in the potentiometric measurement. On the right ordinate the change of the pH is marked under the assumption of a sensitivity of 35 mV/pH. The scale was calibrated to zero at approximately 1 hour 30 minutes when the impedimetric curves change. After approximately two hours there is a peak in the potentiometric signal. An increase corresponds to an acidification as was shown in figure 5.24, which would be expected from the cell apoptosis. Even though the change of the pH is not totally unreasonable it is at the upper limit of what is reported in literature [54]. Another problem are the changes before and after the peak. The other channels did not show this behavior, thus it cannot be an overall effect of the measurement. Nevertheless the time lapse seems promising for future experiments.

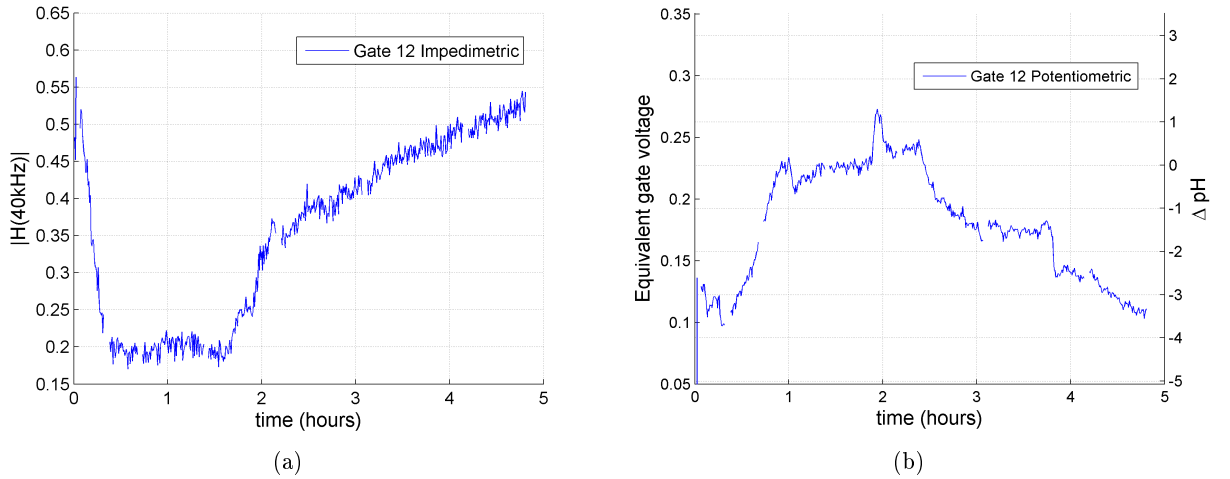


Figure 5.27: Impedimetric (a) and potentiometric (b) measurement of a cell during apoptosis.

5.8 Cell modeling

In the previous section changes in the transfer function of adhered cells, induced by trypsin activity were monitored. The observations showed a shift of the transfer function to higher frequencies which was assigned to the degrading adherence of the cells on the chip. However, it could not be explained why the transfer functions showed this behavior. To clarify the system behavior, a

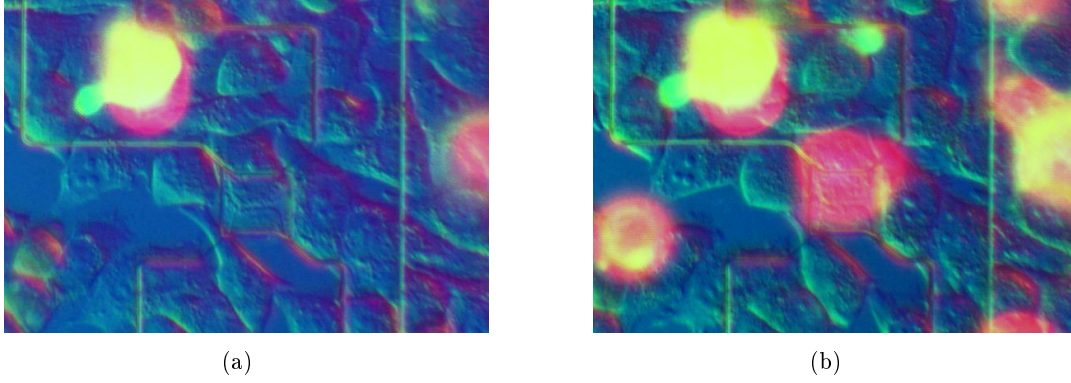


Figure 5.28: Picture with fluorescence of annexin-V and propidium iodide of a cell on gate 12 after 1h30min (a) and after 2h (b) in CPT-11.

model was developed by the same approach which was already presented in section 5.6 for the microwires. The model was fitted to the measured data and by variation of its elements, the system behavior was discussed.

5.8.1 Phenomenological cell description

In figure 5.29(a) the transfer function of an ISFET with an adhered cell is shown. In order to describe it phenomenologically, the same method as in subsection 5.6.2 was used. However, no combination of time constants could be found which would allow a good fit. The steepness of the transfer function was that high that only zero and pole points of very high order ($n \geq 5$) could describe it. However this was unrealistic since a large amount of elements would have been necessary to generate such high order time constants. This was a problem which was encountered on several cell measurements. Therefore a different approach was developed to find a set of suitable time constants.

The shape of the measured curve resembles a band-stop filter which blocks signals in a limited frequency range (in this case between 10 kHz and 1 MHz). To find an appropriate set of time constants, the cell transfer function was handled as if it was such a filter. In this manner it was possible to find a suitable phenomenological description and to introduce a special case of time constants which was not discussed yet.

Band-stop filters can be described by adding the transfer functions of a low- and high pass [32]. These two filters define the pass bands of the overall filter. Only the frequency range between both cut-off frequencies is attenuated. In figure 5.29(a) the process to design a band-stop filter is shown step by step. The cut off frequencies of the low and high pass filters are chosen to match the edges of the band-stop. Obviously the measured data has a higher slope than the edges of the filters. However the sum of both filters shows a good approximation of the

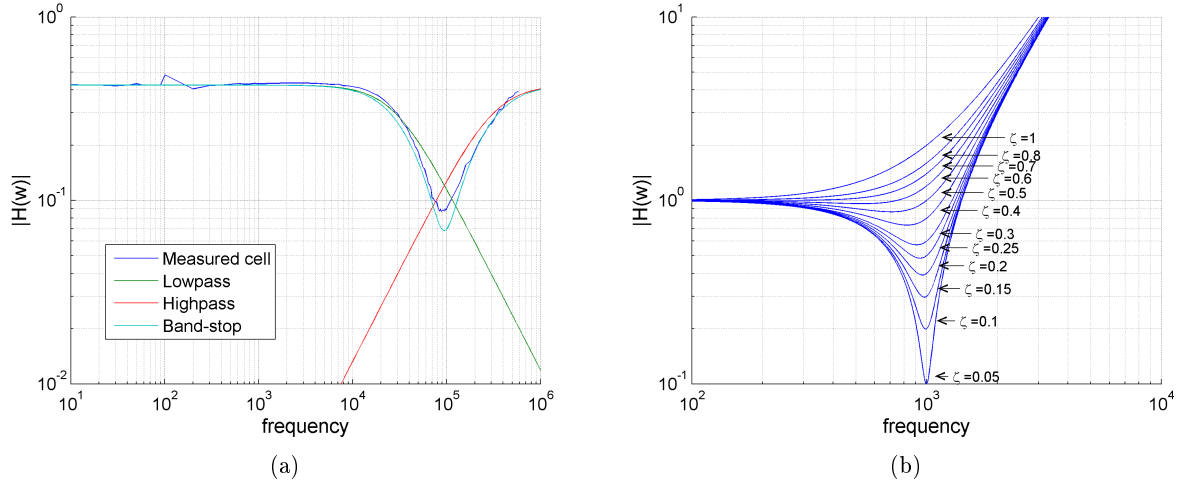


Figure 5.29: **(a)** Retrieval of the transfer function of an ISFET with an adhered cell by combination of a low pass and a high pass filter. **(b)** Influence of ζ on the transfer function of a second order zero point.

transfer function of the cell. The transfer functions of the low pass $H_l(w)$ and of the high pass $H_h(w)$ are given by:

$$H_h(w) = \frac{jw\tau_h}{1 + jw\tau_h} \quad H_l(w) = \frac{1}{1 + jw\tau_l} \quad (5.26)$$

The sum of both filters results in the transfer function of the band-stop $H(w)$:

$$H(w) = H_h(w) + H_l(w) = \frac{1 + jw \cdot 2\tau_h - w^2 \cdot \tau_h\tau_l}{(1 + jw\tau_l) \cdot (1 + jw\tau_h)} \quad (5.27)$$

The right term of equation 5.27 is the normalized transfer function of a band-stop. There are two pole points in the denominator and a special case of a time constant in the numerator. This special time constant can be written more generally as:

$$H_{zp}(w) = 1 + jw \cdot 2\zeta\tau_{n1} - w^2 \cdot \tau_{n1}^2 \quad (5.28)$$

As usual the time constant τ_{n1} determines the location of the zero point. Since the equation contains a square of the frequency, it is a time constant of second order. The difference to a normal time constant consists in the parameter ζ . If it equals one, $H_{zp}(w)$ becomes:

$$H_{zp}(w) = (1 + jw\tau_{n1})^2 \quad \text{with} \quad \zeta = 1 \quad (5.29)$$

If ζ is bigger than one, $H_{zp}(w)$ can be factorized into two terms with separate time constants. However, if ζ is smaller than one, it cannot be factorized any more. In this case $H_{zp}(w)$ is a second order time constants, which shows an overshoot at the cut-off frequency. To clarify this, the magnitude of equation 5.28 is shown in figure 5.29(b) for different values of ζ . The zero point

is set to 1 kHz. The smaller ζ becomes, the higher is the overshoot of the magnitude. For $\zeta = 0$ the curve will diverge. By comparing equation 5.27 and 5.28 τ_{n1} and ζ can be calculated:

$$\tau_{n1} = \sqrt{\tau_h \cdot \tau_l} \quad \zeta = \sqrt{\frac{\tau_h}{\tau_l}} \quad (5.30)$$

To achieve a band-stop characteristic τ_l needs to be larger than τ_h , causing a ζ which is always smaller one. Thus the steep edges of the band-stop and the cell transfer function were due to the overshoot of a time constant. In the following the band-stop transfer function was used to fit the cell measurements. In figure 5.30 the transfer function of an ISFET with a cell immersed in a PBS buffer is shown. It was fitted by the following transfer function and time constants:

$$H(w) = V \cdot \frac{(1 + jw \cdot 2\zeta\tau_{n1} - w^2 \cdot \tau_{n1}^2) \cdot (1 + jw\tau_{n2})}{(1 + jw\tau_{p1}) \cdot (1 + jw\tau_{p2})} \quad (5.31)$$

$$V = 0.4375 \quad \zeta = 0.4 \quad \tau_{n1} = \frac{1}{2\pi \cdot 96.9 \text{ kHz}} \quad \tau_{n2} = \frac{1}{2\pi \cdot (-1.118 \text{ MHz})} \quad (5.32)$$

$$\tau_{p1} = \frac{1}{2\pi \cdot 29.2 \text{ kHz}} \quad \tau_{p2} = \frac{1}{2\pi \cdot 291.9 \text{ kHz}} \quad (5.33)$$

The zero pole τ_{n2} could not be located exactly. As its value indicates, it is outside of the measurement range and in some transfer functions it could not be observed at all. In the following subsection an EEC is presented to map the time constants to a network of resistors and capacitors. For that circuit this zero point was not considered since its behavior was left unclear.

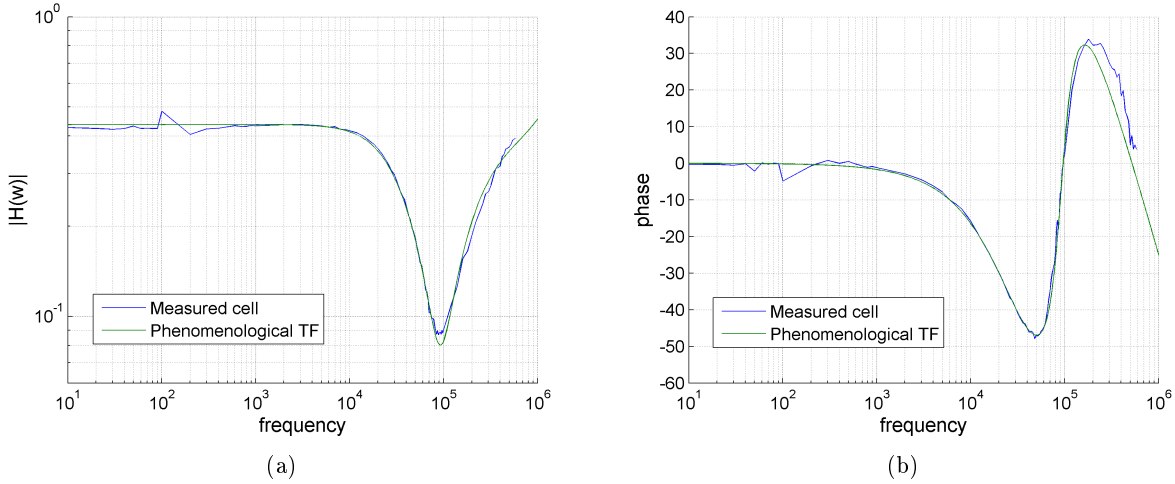


Figure 5.30: Magnitude **(a)** and phase **(b)** of a cell measurement and of its fitted transfer function.

5.8.2 EEC for cell measurements

In order to find a suitable EEC for the biological measurement, the cell itself must be modeled by a set of passive elements. As it was already explained in subsection 2.2.2, the cell membrane can be approximated by a resistor and a capacitor in parallel. This representation is used in figure 5.31 to develop an EEC of an entire cell, adhered on a gate (additional elements describing the sensor are not shown in this figure). A current between the electrolyte and the gate has two possible pathways. It can go through the cell, passing the cell membrane two times. This is modeled by C_{Mx} and R_{Mx} for the membrane and by r_i which is the resistance inside the cell. Alternatively the current can pass the small cleft between the cell and the chip which is modeled by the seal resistance R_{Seal} .

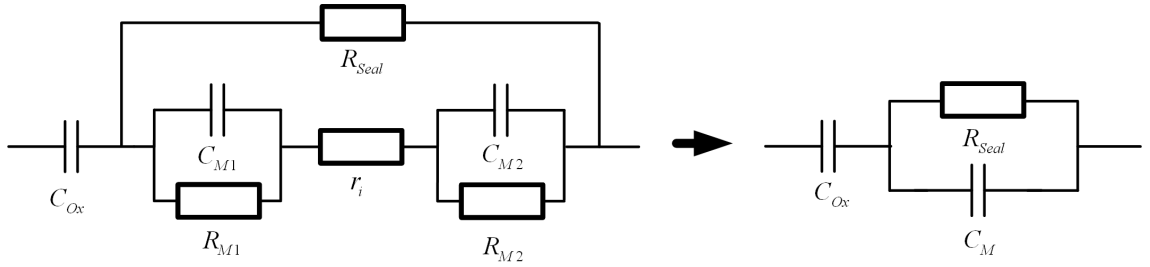


Figure 5.31: Electrical equivalent circuit of a cell adhered to a gate.

This is an EEC which in similar form was already used in other publications [11] to model cell transfer functions. Since under normal circumstances the cell membrane is a very good insulator, the resistances R_{M1} and R_{M2} were neglected. r_i is relatively small and was also neglected. Thus the remaining membrane capacitances can be combined to:

$$C_M = \frac{C_{M1} \cdot C_{M2}}{C_{M1} + C_{M2}} \quad (5.34)$$

In order to use this circuit to describe the cell measurements, it was further extended by the resistance of the electrolyte R_{EL} , the conducting line capacity C_L and the current source $g_m \cdot u_{gs}$ which models the sensor. For a more detailed description it is referred to subsection 5.6.3, where these elements were introduced. The overall EEC is shown in figure 5.32.

As in subsection 5.6.4, the time constants of this model were calculated analytically. In this case the calculation was simpler, since it contains less elements and less factorisation steps were necessary. Nevertheless the solution had to be simplified to limit complexity. Therefore similar assumptions on the elements size were made. In the following the prerequisites for the different elements, the transfer function and the solutions for the time constants are shown:

$$\begin{aligned} R_{Seal}/\Omega &\gg R_{El}/\Omega &\gg g_m/S &\gg C_L/F &\gg (C_{Ox}, C_M)/F \\ 10^6 &10^3 &10^{-5} &10^{-10} &10^{-12} - 10^{-13} \end{aligned} \quad (5.35)$$

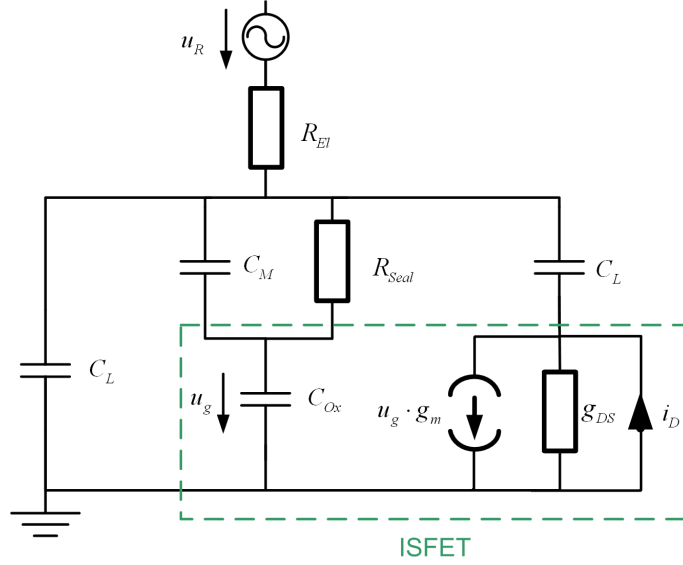


Figure 5.32: Equivalent circuit for cell measurements on ISFET devices.

$$H(w) = R_A \cdot g_m \cdot \frac{1 + jw \cdot 2\zeta\tau_{n1} - w^2 \cdot \tau_{n1}^2}{(1 + jw\tau_{p1}) \cdot (1 + jw\tau_{p2})} \quad (5.36)$$

$$\tau_{n1} = \sqrt{\frac{1}{g_m} \cdot R_{Seal} C_L (C_M + C_{Ox})} \quad \zeta = \frac{g_m C_M R_{Seal} + C_L}{2 \cdot \sqrt{g_m R_{Seal} C_L \cdot (C_M + C_{Ox})}} \quad (5.37)$$

$$\tau_{p1} = R_{Seal} \cdot (C_M + C_{Ox}) \quad \tau_{p2} = 2R_{EL} \cdot C_L \quad (5.38)$$

These time constant were used to calculate the elements of the EEC. In the system of equations there are five known values (V , ζ , τ_{n1} , τ_{p1} , τ_{p2}) and six unknown values (C_{Ox} , R_{Seal} , C_M , C_L , g_m , R_{EL}). Additionally R_A is $5 \text{ k}\Omega$ in the TTF-Box). To find a solution one unknown parameter had to be chosen. Therefore, the gate capacitance was favorable since it could be easily estimated. For an ISFET with a gate length of $4\mu\text{m}$, a width of $12\mu\text{m}$ and an gate oxide thickness of 2 nm , the capacitance was approximated by:

$$C_{Ox} = \epsilon_0 \cdot \epsilon_{\text{SiO}_2} \cdot \frac{4 \mu\text{m} \cdot 12 \mu\text{m}}{2 \text{ nm}} \approx 0.807 \text{ pF} \quad \epsilon_{\text{SiO}_2} = 3.8 \quad (5.39)$$

As an example, the elements of the EEC were calculated for the measurement shown in figure 5.30 :

$$\begin{aligned}
g_m &= 85.84 \mu S \\
C_L &= 42.52 pF \\
R_{EL} &= 6.4 k\Omega \\
R_{Seal} &= 5.78 M\Omega \\
C_M &= 0.142 pF
\end{aligned}$$

The transconductance is usually around $100 \mu S$ for our ISFET devices. C_L is lower than the anticipated $150 pF$ calculated in [16]. This could be due to the encapsulation, since parts of the chip were covered by the carrier and as such could not contribute to the capacitance. R_{EL} is in a meaningful range for PBS solution ($\delta_{PBS} \approx 100 \Omega cm$). The value of $5.8 M\Omega$ is quite high for the seal resistance, since it is specified in literature as being around $1 M\Omega$ for HEK293 cells on a PLL layer [22]. However, for this fit a cell which showed an exceptional strong adhesion was chosen which might explain the discrepancy. Generally a cell has a specific membrane capacitance of $C_{mem} = 1 \mu F cm^{-2}$ [31]. For HEK cells on a PLL layer the ratio of the adhered surface, to the remaining surface has been determined to be $\alpha_{JM} = 44.5\%$ [56]. For a typical cell with a diameter of $30 \mu m$ the membrane capacitance can be approximated to:

$$C_{M1} = C_{mem} \cdot \pi r^2 = 7.069 pF \quad C_{M2} = C_{mem} \cdot \frac{\pi r^2}{\alpha_{JM}} = 15.88 pF \quad (5.40)$$

By equation 5.34 the overall capacitance of the membrane can be calculated to $C_{cell} = 4.89 pF$ which is bigger than the calculated C_M . However, the gate surface is only in contact with a small part of the cell. Therefore the entire capacitance of the cell membrane cannot be measured and C_M should always be smaller than C_{cell} . If not the surface of the cell but of the gate is multiplied with the specific membrane capacitance C_{M1} decreases to $0.24 pF$.

The calculations showed that all elements were in a meaningful range and the prefixes of the values were correct. This suggests that the model is suitable for the measured transfer functions and that all assumptions were made correctly. This could also explain, why the elements of the microwires caused problems for modeling. Both EECs of the ISFET and microwires are very similar. However, the calculations differ, since for the cell measurement a time constant with an overshoot was anticipated. This could also be the case for the microwires. For a value of ζ very close to one, the overshoot is not distinguishable, but the assumption of distinct time constants would be wrong. This could lead to the negative prefixes of some elements.

5.8.3 Sensitivity analysis

Sometimes when the curves were fitted, several sets of phenomenological parameters would provide a good agreement. However, not every fit provided good values for the elements of the EEC. For example it proved to be reasonable to choose ζ as big as possible, since too small values would

lead to unrealistic values for C_M . Another important parameter is the gate capacitance C_{Ox} . It must be chosen very carefully since it has a big influence on the transfer function. To illustrate this the deduced values of the elements in the previous subsection were used to calculate the transfer function. Only the parameter C_{Ox} was varied over a wide range to show its influence. The result is shown in figure 5.33(a), the arrows mark the exponent of the capacitance value. By increasing C_{Ox} , the transfer function becomes steeper and is shifted to lower frequencies. This is favorable since the interesting part of the transfer function then shifts to the middle of the measurement range.

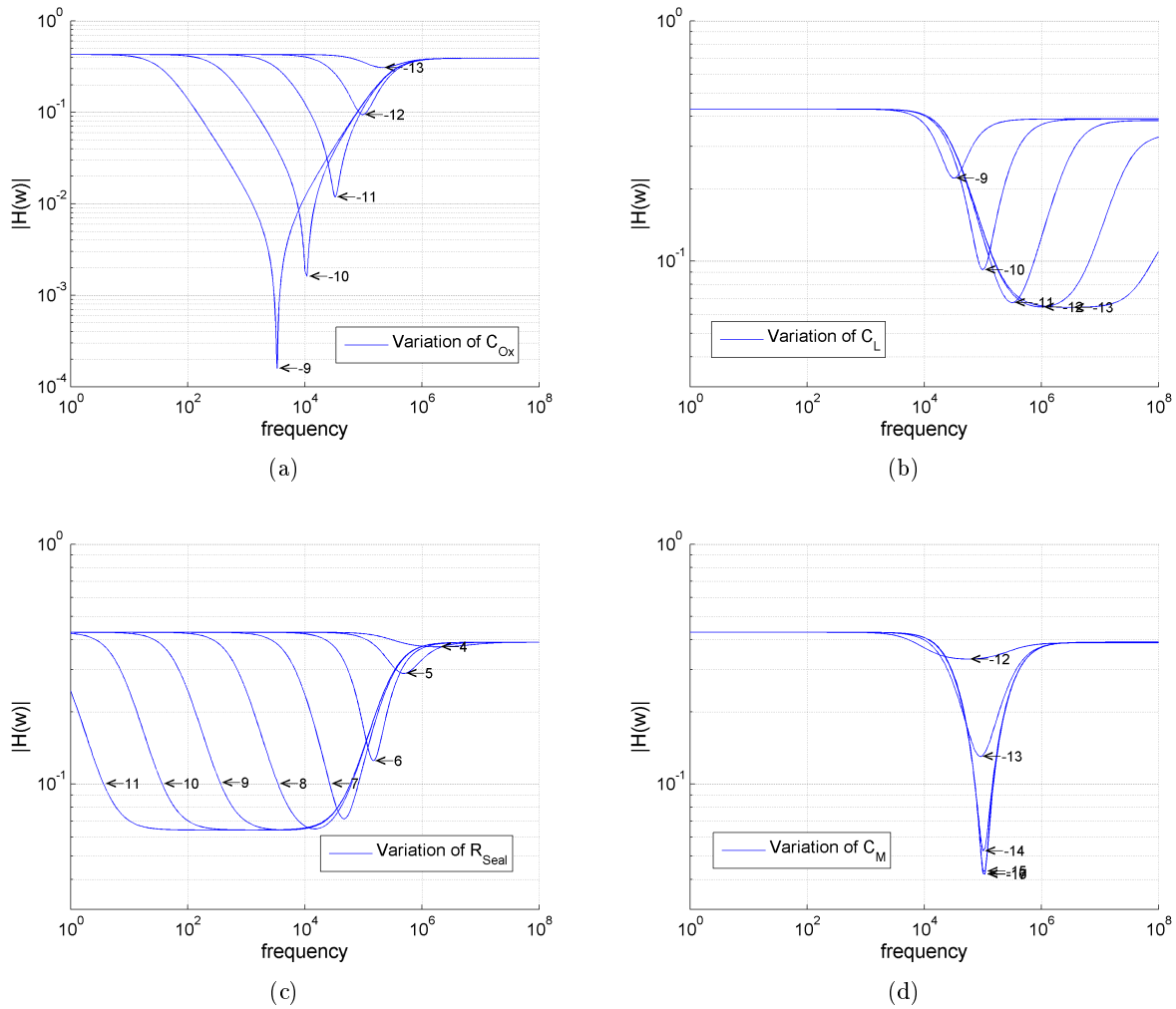


Figure 5.33: Sensitivity analysis of the cell EEC by variation of C_{Ox} (a), C_L (b), R_{Seal} (c) and C_M (d). The values in the figure show the decade of the varied parameter while the other values were taken from the calculated elements in subsection 5.8.2.

Another interesting parameter for the design of the sensors is the conducting line capacitance. In figure 5.33(b) it was varied in a similar way. It caused contrary effects in comparison to the gate capacitance, since the band-stop became bigger when it decreased. However, some time constants were shifted out of the measurement range which should be avoided. Therefore, it seems favorable to increase C_L and C_{Ox} at the same time. Thus the time constants are shifted to lower frequencies and the magnitude does not become too steep.

The same analysis was also performed to understand the changes induced by the seal resistance and the membrane capacitance. In figure 5.33(c) the seal resistance is varied over a wide range. For very high seal resistances, the band-stop region widens to lower frequencies. However for HEK 293 cells the seal resistances will always stay in the range of $10^5 - 10^6$. In contrast erythrocytes on PLL coated chips should show a very wide stop-band, since they have a very high seal resistance of around $100\text{ M}\Omega$ [22]. Changes in the membrane capacitance only affected the steepness of the transfer function. As shown in figure 5.33(d) the changes converged against a minimum value for very small capacitances.

The analysis of the different elements of the transfer function gives a better understanding of the measurement processes. By variations of the model, predictions about the behavior of bioelectronic systems and the future design of biosensors could be made.

Chapter 6

Conclusion and outlook

6.1 Conclusion

In the scope of this thesis concepts and methods to improve signal acquisition, processing and evaluation of bioelectronic sensors were developed. A new amplifier was designed with the aim to minimize the analog components. Since the workload of the signal processing was shifted to the digital side, the analog electronics could be significantly simplified. Their simple design makes the system very scalable up to numerous channels in parallel and it allows the use of different measurement methods with the same hardware. In this context special attention was drawn to transfer function measurements. They can either be measured by the Lock-In or by the MLS approach.

The MLS offers fast measurements with the disadvantage of a low noise rejection. In contrast, the Lock-In provides highly adjustable noise attenuation, but it has some inherent speed limitations. Nevertheless it showed very good measurement results for ISFETs and microwires and can be used in a wide frequency range. Both methods were further extended to allow the monitoring of time resolved changes in the transfer functions and in the constant component. Therefore, they repeatedly perform in parallel potentiometric and impedimetric measurements at a fixed time rate. Since different effects are assigned to the measurands, various processes can be observed and distinguished over a long measurement time.

The system was used to estimate the noise power of the sensor devices. The signal-to-noise ratio was analyzed as a function of the operating point for microwires and ISFETs. The results were compared and showed a similar signal-to-noise ratio and a lower transconductance for the microwires devices. Nanowires will probably have a worse noise characteristic than microwires. However, since the Lock-In offered very good results at the noise level of ISFETs and microwires, it can be anticipated that its noise rejection will also be sufficient for future nanowire devices.

The tools developed in this thesis were used to examine the analog electronics of the amplifier and to find and quantify error sources in the transfer function measurements. This knowledge about the electronics enabled the compensation of errors by software modifications and to sig-

nificantly improve the performance of the system. A noise analysis of the signal source led to improvements of the source quality and made the analysis of the spectral power density of sensor signals possible. By using more precise sampling hardware and by evaluating high amounts of data, the precision of all measurements, especially of the potentiometric ones, could be improved.

Two possible bioelectronic applications of ISFETs with the new amplifier system, which have been examined in the scope of this work, are the examination of cell apoptosis and cell adhesion. Different stages of apoptosis like cell shrinkage, separation and acidification were observed. For cell adhesion it could be shown that cells have a significant influence on measured transfer functions. Based on the transfer functions, it is even possible to estimate, how well a single cell is adhered to a biosensor. A model was developed to map the measured data to meaningful elements of an equivalent electrical circuit. The properties of the cell are described by simple electrical elements, and the values can be calculated by fitting the measured data to the model. As a prerequisite only well-known properties of the biosensor (like the gate capacitance for an ISFET) have to be known. In comparison to optical methods for observation of cell adhesion or apoptosis, this bioelectronic approach additionally quantifies changes in the adhesive properties of the cell and gives further information for interpretation of the involved processes.

A similar model was developed for microwire devices. As in the case of ISFETs, transfer functions were used to deduce the elements of an equivalent electrical circuit. The results showed good agreement to measurements with an external resistance meter. In comparison to ISFETs, microwires show more complex impedance characteristics. The model for microwire transfer functions has more time constants in the measurement range and must be described by more elements. Nevertheless, the model can reliably describe the microwire behavior and be used to explain the results of future experiments.

6.2 Outlook

A drawback of the Lock-In is its limited speed, which causes problems in time critical measurements. Fast changes of the measurands cannot always be monitored because of a long measurement duration, which is determined by the filter efficiency. In this context the filter efficiency is determined by the number of samples needed to attain a high attenuation. This could be improved by using other approaches like FIR or Tschebyscheff filters, which have a very high slope and are very suitable for the Lock-In. Thus, the measurement speed could be improved without a loss of filter attenuation. Especially the results of time series measurements would benefit from such improvements.

The models of the microwire and ISFET devices can be further improved. In the case of microwires the problem of the elements with negative prefix could be solved by assuming time constants which have a low but existing overshoot. This would replace the approximation by normal time constants and explain the negative prefixes.

Because of its flexible design, the developed amplifier is suitable for numerous future applications. The high speed of the MLS enables the readout of large arrays of sensors in a reasonable time. This would allow to electrically monitor not only properties of single cells, but of many cells at the same time in large cell cultures. Especially phenomenological screening with statistical analysis of cell health parameters could profit from such cell culture monitoring.

A way to further increase the available information about various cell parameters is to improve the monitoring of several measurands at a time. With only slight modifications, the system could record the transfer function, constant signal component, SNR and power spectrum simultaneously, enabling a more detailed description of changes in biomolecular or cellular systems. For example, a noise component which is associated to the seal resistance in cell-device coupling is described in literature. By measuring the transfer function and the noise at the same time, the seal-associated noise could be compared to the seal resistance deduced of the model developed in this thesis.

Furthermore the time series measurements could be synchronized for an automatic image acquisition of cells. The combination with optical information could be used for the verification of the electrically measured data and to find out which effects can be observed more precisely optically or electrically.

The modeling of the sensor devices leaves space for future analysis and could be used to support the design of novel sensors. Only elements which contribute to time constants in the measurement range can be observed by impedimetric measurements. For the design of novel devices it might be interesting to adjust the dimension of sensor specific elements to move single time constants into or outside of the measurement range. This way theoretically predicted elements could become measurable or in contrary, the influence of unwanted elements could be eliminated. This would be interesting to avoid the influence of the electrolyte resistance on DNA-hybridization measurements. Furthermore, in order to verify the models developed in this thesis, measurements varying only single elements in the system should be performed. For example by using polyelectrolytes [57], single layers of molecules can be deposited on microwires. These layers can be directly assigned to elements in the model and as such be used for its verification.

The most important application of the amplifier system will be bioelectronic measurements with nanowire devices. Because of its scalability and its flexible design, it should be possible to perform these measurements, even if device characteristics like noise generation degrade by a factor of ten in comparison to microwires. Since the amplifier can also be used with ISFETs, it forms an ideal tool for exact comparisons of these two types of devices.

Appendix A

MLS proof

The equation A.1, used in subsection 3.3.2, should be proven to be correct. In the following $m(k)$ will be a valid M-sequence as defined in subsection 3.3.1.

$$\sum_{k=-\infty}^{\infty} y(k) = - \sum_{k=-\infty}^{\infty} h(k) \quad (\text{A.1})$$

With the relation $y(k) = m(k) * h(k)$ the sum of $y(k)$ can be written as:

$$\sum_{k=-\infty}^{\infty} y(k) = \sum_{k=-\infty}^{\infty} m(k) * h(k) \quad (\text{A.2})$$

$$\begin{aligned} \sum_{k=-\infty}^{\infty} y(k) &= \sum_{k=-\infty}^{\infty} \sum_{n=-\infty}^{\infty} m(n) \cdot h(k-n) \\ &= \sum_{n=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} m(n) \cdot h(k-n) \\ &= \sum_{n=-\infty}^{\infty} m(n) \left(\sum_{k=-\infty}^{\infty} h(k-n) \right) \\ &= \left(\sum_{n=-\infty}^{\infty} m(n) \right) \cdot \left(\sum_{k=-\infty}^{\infty} h(k) \right) \end{aligned}$$

Since a valid M-sequence always contains a -1 more than $+1$, the sum of $m(k)$ is always -1 . This results in:

$$\sum_{k=-\infty}^{\infty} y(k) = - \sum_{k=-\infty}^{\infty} h(k) \quad (\text{A.3})$$

Appendix B

ISFET EEC

In this chapter the transfer function of the cell measurement EEC presented in subsection 5.8.2 is calculated. The goal is to find a normalized solution for $H(jw)$ and to separate its time constants. By several approximations these should be simplified as much as possible. In figure B.1 the EEC is shown again with some additional helper variables. For an explanation of the EEC please refer to subsection 5.8.2 and 5.6.3.

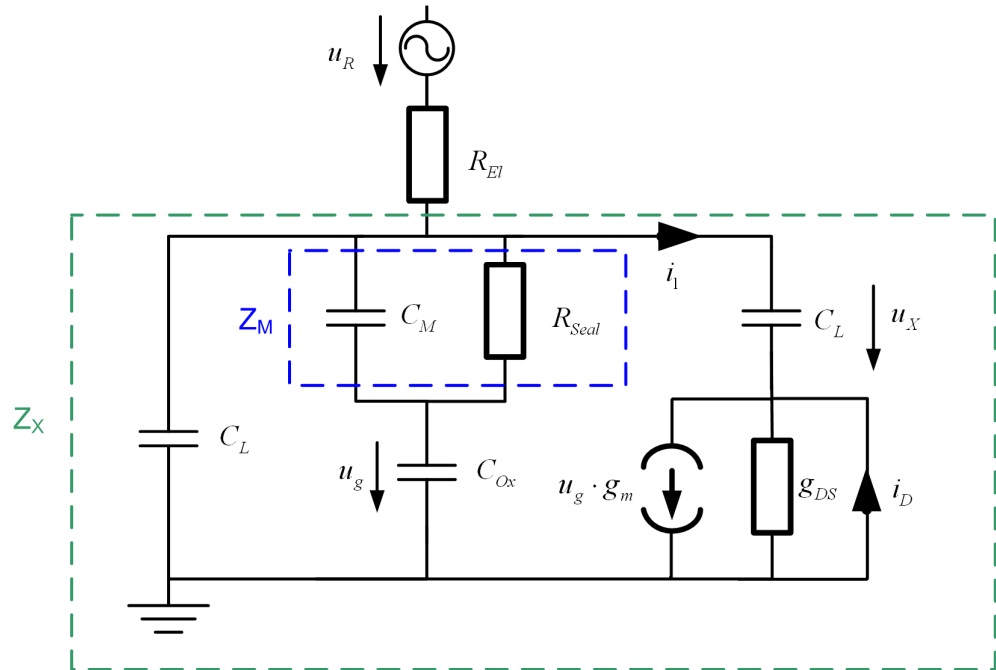


Figure B.1: EEC which describes an adhered cell on a sensor.

At first some basic equations can be deduced out of the EEC:

$$i_D = g_m \cdot u_g - i_1 \quad i_1 = u_x \cdot jwC_L \quad (\text{B.1})$$

$$u_g = \frac{u_x}{1 + jwC_{Ox}Z_M} \quad u_x = \frac{u_R}{1 + \frac{R_{EL}}{Z_X}} \quad (\text{B.2})$$

$$Z_M = \frac{R_{Seal}}{1 + jwC_M \cdot R_{Seal}} \quad Z_X = \frac{1}{jw2C_L + \frac{1}{Z_M + \frac{1}{jwC_{Ox}}}} \quad (\text{B.3})$$

A transfer function is defined by an output to input voltage ratio. The output voltage of the amplifier can be calculated by $u_O = R_A \cdot i_D$. R_A is the amplification of the impedance converter (see subsection 5.4.3). Thus $H(jw)$ can be written as:

$$H(jw) = \frac{u_O}{u_R} = \frac{R_A \cdot i_D}{u_R} = \frac{R_A}{u_R} \cdot (g_m \cdot u_g - i_1) \quad (\text{B.4})$$

By the equations B.1 - B.3 $H(jw)$ is extended to:

$$H(jw) = \frac{R_A}{u_R} \cdot \left(g_m \cdot \frac{u_x}{1 + jwC_{Ox}Z_M} - u_x \cdot jwC_L \right) \quad (\text{B.5})$$

$$H(jw) = R_A \cdot \left(\frac{g_m}{1 + \frac{jwC_{Ox} \cdot R_{Seal}}{1 + jwC_M \cdot R_{Seal}}} - jwC_L \right) \cdot \frac{1}{1 + R_{EL} \cdot \left(jw2C_L + \frac{1}{Z_M + \frac{1}{jwC_{Ox}}} \right)} \quad (\text{B.6})$$

Then $H(jw)$ is normalized to:

$$H(jw) = R_A \cdot g_m \cdot \frac{1 + jwA_0 - w^2B_0}{1 + jwC_0 - w^2D_0} \quad (\text{B.7})$$

$$A_0 = C_MR_{Seal} + \frac{C_L}{g_m} \quad B_0 = \frac{C_LR_{Seal}}{g_m}(C_M + C_{Ox}) \quad (\text{B.8})$$

$$C_0 = R_{Seal}(C_{Ox} + C_M) + R_{EL}(2C_L + C_{Ox}) \quad (\text{B.9})$$

$$D_0 = R_{Seal}R_{EL}(2C_L(C_{Ox} + C_M) + C_{Ox}C_M) \quad (\text{B.10})$$

A_0 and B_0 are used to calculate τ_{n1} and ζ :

$$\tau_{n1} = \sqrt{\frac{R_{Seal}C_L}{g_m}(C_M + C_{Ox})} \quad (\text{B.11})$$

$$\zeta = \frac{A_0}{2\tau_{n1}} = \frac{g_mC_MR_{Seal} + C_L}{2 \cdot \sqrt{g_mR_{Seal}C_L \cdot (C_M + C_{Ox})}} \quad (\text{B.12})$$

However the pole points have to be factorized. This can be performed by:

$$(1 + jwC_0 - w^2D_0) \stackrel{!}{=} (1 + jw\tau_{p1}) \cdot (1 + jw\tau_{p2}) \quad (\text{B.13})$$

$$\tau_{p1} = \frac{C_0 \pm \sqrt{C_0^2 - 4D_0}}{2} \quad \tau_{p2} = \frac{2D_0}{C_0 \pm \sqrt{C_0^2 - 4D_0}} \quad (\text{B.14})$$

By back substitution the pole points become very complex equations. However not all terms are of importance. Under the following prerequisite they can be simplified to:

$$\begin{array}{ccccccccc} R_{Seal}/\Omega & \gg & R_{El}/\Omega & \gg & g_m/S & \gg & C_L/F & \gg & (C_{Ox}, C_M)/F \\ 10^6 & & 10^3 & & 10^{-5} & & 10^{-10} & & 10^{-12} - 10^{-13} \end{array} \quad (\text{B.15})$$

$$\tau_{p1} = R_{Seal}(C_M + C_{Ox}) \quad \tau_{p2} = 2R_{EL}C_L \quad (\text{B.16})$$

Appendix C

Microwire EEC

In this chapter the transfer function of the microwire EEC presented in chapter 5.6.3 is calculated. The calculation is similar to the arithmetic in appendix B. In figure C.1 the EEC is shown again with some additional helper variables. For an explanation of the EEC please refer to chapter 5.6.3.

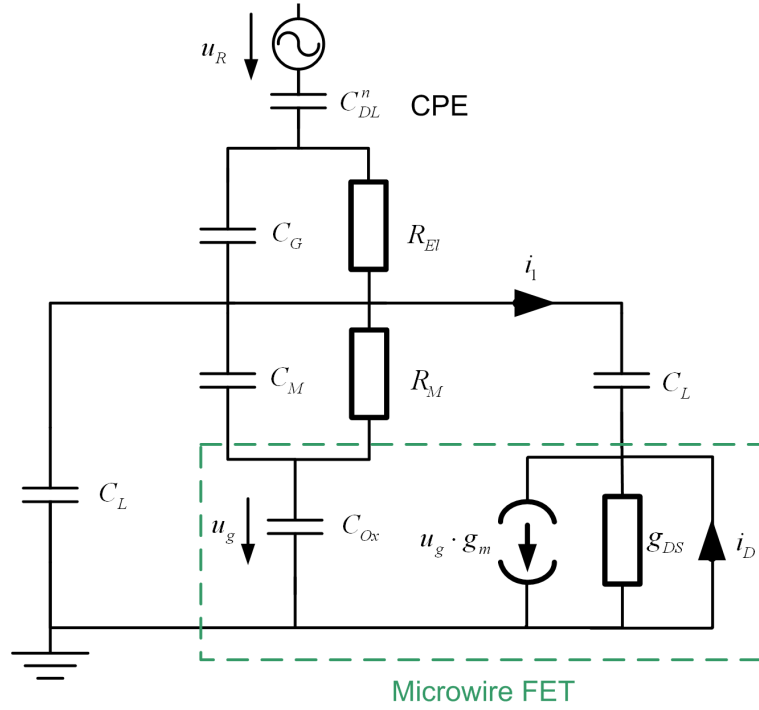


Figure C.1: EEC which is used to fit the microwire measurements.

At first some basic equations out of the EEC can be found:

$$i_D = g_m \cdot u_g - i_1 \quad i_1 = u_x \cdot j\omega C_L \quad (C.1)$$

$$u_g = \frac{u_x}{1 + j\omega C_{Ox} Z_M} \quad u_x = \frac{u_R}{1 + \frac{Z_{EL}}{Z_X}} \quad (C.2)$$

$$Z_{EL} = \frac{1}{j\omega^n C_{DL}^n} + \frac{R_{EL}}{1 + j\omega R_{EL} C_G} \quad Z_M = \frac{R_M}{1 + j\omega C_M \cdot R_M} \quad (C.3)$$

$$Z_X = \frac{1}{j\omega 2C_L + \frac{1}{\frac{1}{j\omega C_{Ox}} + \frac{1}{1 + j\omega C_M R_M}}} \quad (C.4)$$

A transfer function is defined by an output to input voltage ratio. The output voltage of the amplifier can be calculated by $u_O = R_A \cdot i_D$. R_A is the amplification of the impedance converter (see chapter 5.4.3). Thus $H(j\omega)$ is written as:

$$H(j\omega) = \frac{u_O}{u_R} = \frac{R_A \cdot i_D}{u_R} = \frac{R_A}{u_R} \cdot (g_m \cdot u_g - i_1) \quad (C.5)$$

By the equations C.1 - C.4 $H(j\omega)$ is extended to:

$$H(j\omega) = \frac{R_A}{u_R} \cdot \left(g_m \cdot \frac{u_x}{1 + j\omega C_{Ox} Z_M} - u_x \cdot j\omega C_L \right) \quad (C.6)$$

$$H(j\omega) = R_A \cdot \underbrace{\left(\frac{g_m}{1 + \frac{j\omega C_{Ox} \cdot R_M}{1 + j\omega C_M \cdot R_M}} - j\omega C_L \right)}_{H_1(j\omega)} \cdot \frac{1}{1 + \frac{Z_{EL}}{Z_X}} \quad (C.7)$$

$$H(j\omega) = R_A \cdot H_1(j\omega) \cdot \underbrace{\frac{1}{1 + \left(\frac{1}{j\omega^n C_{DL}^n} + \frac{R_{EL}}{1 + j\omega R_{EL} C_G} \right) \cdot \left(j\omega 2C_L + \frac{1}{\frac{1}{j\omega C_{Ox}} + \frac{1}{1 + j\omega C_M R_M}} \right)}}_{H_2(j\omega)} \quad (C.8)$$

The next step is to factorise $H(j\omega)$. To improve readability the problem is separated in $H_1(j\omega)$ and $H_2(j\omega)$.

$$H_1(j\omega) = \frac{g_m \cdot (1 + j\omega R_M \cdot C_M)}{1 + j\omega R_M \cdot (C_{Ox} + C_M)} - \frac{j\omega C_L - \omega^2 R_M C_L \cdot (C_{Ox} + C_M)}{1 + j\omega R_M \cdot (C_{Ox} + C_M)} \quad (C.9)$$

$$H_1(j\omega) = g_m \cdot \frac{\overbrace{1 + j\omega \left(R_M \cdot C_M - \frac{C_L}{g_m} \right)}^{A_0} + \omega^2 \overbrace{\frac{C_L \cdot R_M}{g_m} (C_{Ox} + C_M)}^{B_0}}{1 + j\omega R_M \cdot (C_{Ox} + C_M)} \quad (C.10)$$

In $H_1(jw)$ only the numerator needs to be factorized. The same approach as in appendix B (see equation B.14) is used.

$$(1 + jwA_0 - w^2B_0) \stackrel{!}{=} (1 + jw\tau_{n1}) \cdot (1 + jw\tau_{n2}) \quad (C.11)$$

$$\tau_{n1} = \frac{A_0 \pm \sqrt{A_0^2 - 4B_0}}{2} \quad \tau_{n2} = \frac{2B_0}{A_0 \pm \sqrt{A_0^2 - 4B_0}} \quad (C.12)$$

The resulting solutions for τ_{n1} and τ_{n2} are too complex. Therefore the following dimensions of the elements is assumed:

$$\begin{array}{ccccccccc} R_M/\Omega & \gg & R_{El}/\Omega & \gg & g_m/S & \gg & C_L/F & \gg & (C_{Ox}, C_M, C_G, C_{DL})/F \\ 10^{13} & & 10^6 - 10^3 & & 10^{-5} & & 10^{-10} & & 10^{-12} - 10^{-13} \end{array} \quad (C.13)$$

They allow to simplify τ_{n1} and τ_{n2} :

$$\tau_{n1} = C_M \cdot R_M \quad \tau_{n2} = -C_L \cdot \frac{C_M + C_{Ox}}{g_m \cdot C_M} \quad (C.14)$$

To factorize $H_2(jw)$ is more complex. By expanding the term it is written as:

$$\frac{1}{H_2(jw)} = 1 + \frac{(1 + jwR_{EL}C_G + jw^n C_{DL}^n R_{EL}) \cdot (jw(C_{Ox} + 2C_L) - w^2(C_{Ox}C_MR_M + 2C_LR_M(C_M + C_{Ox}))}{jw^n C_{DL}^n (1 + jwR_{EL}C_G) \cdot (1 + jwR_M(C_M + C_{Ox}))} \quad (C.15)$$

Although $H_2(jw)$ is not fully normalized yet, the right term of the equation provides some new time constants. Since $H_2(jw)$ is multiplied with $H_1(jw)$ to get the overall transfer function, the term $(1 + jwR_M \cdot (C_{Ox} + C_M))$ is canceled out. $(1 + jwR_{EL}C_G)$ provides an additional zero point:

$$\tau_{n3} = R_{EL} \cdot C_G \quad (C.16)$$

When $H_2(jw)$ is normalized its numerator will have the following form:

$$-jw \cdot (1 + jwX_1 - w^2X_2 + w^{n-1}X_3 + jw^nX_4 - w^{n+1}X_5) \quad (C.17)$$

A suitable ansatz to factorize this polynom would be:

$$-jw \cdot (1 + jwA) \cdot (1 + jwB) \cdot \left(1 + jw^{\frac{n-1}{2}}C\right) \cdot \left(1 - jw^{\frac{n-1}{2}}C\right) = \quad (C.18)$$

$$-jw \cdot (1 + jw \cdot (A + B) - w^2 \cdot A \cdot B + w^{n-1} \cdot C^2 + jw^n C^2 \cdot (A + B) - w^{n+1} \cdot ABC^2) \quad (C.19)$$

By comparing equation C.17 and C.19 the following relations are found:

$$X_1 = A + B \quad X_2 = A \cdot B \quad X_3 = C^2 \quad X_4 = C^2(A + B) \quad X_5 = ABC^2 \quad (\text{C.20})$$

Thus A , B and C can be determined by:

$$A = \frac{X_1}{1 + X_2} \quad B = \frac{X_1}{1 + \frac{1}{X_2}} \quad C = \sqrt{X_3} \quad (\text{C.21})$$

However, the following two equations have to be fulfilled:

$$X_4 = X_3 \cdot X_1 \quad X_5 = X_2 \cdot X_3 \quad (\text{C.22})$$

Unfortunately, both equations cannot be fulfilled. Nevertheless the time constant A , B and C were calculated. For a set of given elements values, the results of the exact solution of the EEC and the approximated time constants were compared. This showed, that C time constant is negligible probably because $w^{\frac{n-1}{2}} \rightarrow w^0$. The time constant B showed no analogy to the transfer functions of the exact solution. This is the pole point of the microwire EEC which could not be deduced, because both equations in C.22 could not be fulfilled. However, A showed good agreement and by using the simplifications in equation C.13 it could be approximated by:

$$\tau_{p1} = A = R_M(C_{Ox} + C_M) \quad (\text{C.23})$$

Appendix D

Composition of buffer solutions

Phosphate buffered saline (PBS)

Component	Concentration
NaCl	137 mM
Phosphate	10 mM
KCL	2.7 mM

Table D.1: Composition of PBS.

Solution used for apoptosis measurements

Component	Amount
Annexin-V binding buffer	1 ml
Annexin-V	50 μ l
Propidium iodide buffer	100 μ l
Irinotecan hydrochloride CPT-11 0.5 mg/ml	6 μ l

Table D.2: Composition of buffer used in apoptosis measurements.

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