Lappeenranta University of Technology Faculty of Technology Degree Program in Electrical Engineering

Master's Thesis

Joonas Talvitie

ELECTRICAL EQUIVALENT CIRCUIT FOR DRY BACTERIORHODOPSIN SENSOR

Examiners: Professor Pertti Silventoinen Lasse Lensu, Docent, D.Sc. (Tech.)

Supervisor: Mikko Kuisma D.Sc. (Tech.)

ABSTRACT

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Keywords: bacteriorhodopsin, electrical equivalent circuit, photoelectric response, measurement instrumentation

Bacteriorhodopsin (BR) is a photosensitive protein which functions as a light-driven proton pump. Due to its photoactivity, BR could be used in photosensing and information processing which has inspired researchers to study the photoelectric response and the appropriate measurement instrumentation for BR.

In this thesis, the measurement instrumentation connected to a dry BR sensor was confirmed to affect the photovoltage response measured by using voltage amplifiers. Changing of the input impedance of the measurement instrumentation was shown to alter a part of the measured photovoltage response. The photocurrent measurements using transimpedance amplifier and the presented electrical equivalent circuit were used to show that the photocurrent measurements have no significant effect on the photoelectric response.

The photocurrent was shown to be a derivate of the photovoltage response measured from the dry BR sensor when it was compared to the response measured with a voltage amplifier. This confirmed that another part of the photovoltage response was not affected by the measurement instrumentation. The time-variant behavior of the dry BR sensor was confirmed in both the photocurrent and the photovoltage measurements. This was caused by the fact that the capacitance of the dry BR sensor changes with the excitation light intensity.

TIIVISTELMÄ

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Joonas Talvitie

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Bakteerirodopsiini on valoon reagoiva proteiini, joka toimii luonnossa protonipumppuna. BR:n valoaktiivisuuden takia sitä voitaisiin käyttää tietojenkäsittelyssä, mikä on innostanut tutkimaan BR:n valosähköistä vastetta ja sen mittauslaitteistoa.

Tässä diplomityössä mittauslaitteiston havaittiin vaikuttavan kuivalta BR sensorilta mittavaan valosähköiseen vasteeseen. Mittauslaitteiston tuloimpedanssia muuttamalla havaittiin myös muutos osassa mitattua jännitevastetta. Transimpedanssivahvistimella tehtyjä virtamittauksia ja esitettyä sähköistä sijaiskytkentää käyttäen todistettiin, että virtamittaukset eivät vääristä kuivalta BR sensorilta mitattavaa signaalia.

Virran osoitettiin olevan sensorilta mitattavan jännitteen aikaderivaatta vertaamalla sitä jännitevahvistimelta saatuihin tuloksiin. Tämä myös varmisti, ettei mittauslaitteiston tuloimpedanssi vaikuta kuin osaan mitattua jännitevastetta. Kuivan BR sensorin aikavarianttikäyttäytyminen havaittiin sekä virrassa että sensorilta mitattavassa jännitteessä. Tämän vahvistettiin johtuvan siitä, että kuivan BR sensorin kapasitanssi muuttuu valoärsykkeen intensiteetin mukaan.

PREFACE

This master's thesis continues the bachelor's thesis I started during autumn 2009 at the Laboratory of Applied Electronics, Department of Electrical Engineering, Lappeenranta University of Technology, Finland. During the last few years, a huge amount of work has been done for this thesis, but this would have not been possible without the work done before me concerning the topic. Fortunately, I had support which deserves thanks.

Most of all, I want to thank my supervisor Mikko Kuisma for the guidance in the academic research during the bachelor's thesis and now with the master's thesis. I also want to thank Docent Lasse Lensu from Department of Information Technology for giving another perspective to the research and guidance during the thesis. I want to thank Professor Pertti Silventoinen for reviewing this thesis and giving comments concerning the details of it. The support of Toni Kuparinen for Labvision Technologies in assisting with the light sources used in this thesis is also appreciated.

Finally, I thank my family for their support during my studies.

Lappeenranta, December 7th, 2011

Joonas Talvitie

Nomenclature

- ϵ Permittivity of dielectric material
- $\tau_{C1,C2}$ Time constant of photocurrent response
- $\tau_{V1,V2,V3}$ Time constant of photovoltage response
- A Area of the BR sensor's electrodes
- BR Bacteriorhodopsin sensor
- C Capacitance
- $C_{\rm F}$ Feedback capacitor in transimpedance amplifier
- C_{IN} Input capacitance of the measurement instrumentation
- *C*_M Membrane capacitance
- C_{P,ch} Series capacitance, Chemical capacitance
- C_{tot} Total capacitance of the BR sensor and the measurement instrumentation
- *d* Thickness of dielectric material
- GBWP Gain-bandwidth product
- $I_{\rm ph}$ Photocurrent measured from the BR sensor
- *IC* Integrated circuit
- PCB Printed Circuit Board
- *PM* Purple membrane
- *PW* Pulsewidth of the light pulse
- Q_{ph} Light-induced charge
- $R_{\prime\prime}$ DC-resistance
- $R_{\rm F}$ Feedback resistor setting gain in transimpedance amplifier
- R_{IN} Input resistance of the measurement instrumentation
- *R*_M Membrane resistance
- $R_{\rm P}$ Internal resistance of the voltage source generating the photoelectric response

- *R*_s Photoactive DC-resistance
- *RF* Radio frequency
- $SNR\;$ Signal to Noise Ratio
- TIA Transimpedance amplifier
- $U_{\rm ph}$ Photovoltage measured from the BR sensor
- $V_{\rm S}$ Voltage source generating the photoelectric response

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1 INTRODUCTION

1.1 Background

Todays information processing is based mainly on semiconductors but demand for faster and more efficient alternatives is increasing. This is due to the fact that the rate of downscaling semiconductor-based electronic circuits is slowing down. To maintain the rate of performance increase, alternative technologies can be found in nanotechnology. Nanotechnology may enable us to scale circuits beyond the limits of modern transistors with reasonable costs. [1] This has raised an interest towards biomolecules and its possibilities, for example, in information processing [2]. One of the materials studied for information processing is bacteriorhodopsin (BR). Bacteriorhodopsin has been under studying for number of reasons. One of them is photoactivity which could be used, for example, in information processing. BR is a photosensitive protein which functions as a light-driven proton pump. It can be found in the purple membrane (PM) of the archaean Halobacterium salinarum [3].

Large quantities of BR can be found in salt marches [3] and if BR is used, for example, in molecular electronics, it has potential to be economically produced [4]. Other advantages of BR are that it has high quantum efficiency (0.65), it can function in extreme environmental conditions [5, 6, 7, 8] and it can be stored for years [9]. BR has many technical applications, for example, in imaging [10, 11, 12], in protein-based memory [13] and in a bio-photoreceiver [14].

Bacteriorhodopsin is used mostly in technical applications so far as a BR sensor to detect light. The BR sensors are used in both dried state and in suspension [15, 16, 10]. The dry BR sensors, which are used in this thesis, are prepared by mixing PM fragments in a polyvinyl alcohol. The mixture is then spread on a conductive glass. The conductive glass and a thin layer of gold, sputtered on dried BR film, function as electrodes of BR sensor.

Also the loading of the BR sensors has been under intensive studying [17, 18, 16, 19, 12, 20, 21]. This has raised discussion [15, 18, 12] about how the measuring instrumentation for the BR sensor should be chosen and how it affects the measurements. This inspired the cooperation between the departments of Electrical Engineering and Information Technology in Lappeenranta University of Technology ultimately leading to this masters thesis.

1.2 Objectives and Restrictions

The main objective of this thesis is to find an electrical equivalent circuit for dry BR sensor which explains the photoelectric responses received using the amplifiers designed in previous research [10, 19, 20, 21]. Modeling of the photoelectric responses from the BR sensor is done using electrical equivalent circuit, not by exponential fitting or estimation of induced charges.

Published electrical equivalent circuits and measurement methods used by the other research groups, are reviewed. Designing the electrical equivalent circuit is done based on the literature and the measured photocurrent and photovoltage responses using the designed amplifiers. Photoelectric responses consisting of charge movement, photovoltage and photocurrent responses are not explained using chemical reactions or photocycle of BR. The hypotheses about the proposed electrical equivalent circuit is experimentally tested using the measured photocurrent and photovoltage responses and compared to the results published by the other research groups. The experimental results are compared to simulated results obtained using a simulation model of the electrical equivalent circuit of BR.

Partial goal of the research is to discuss limitations of the designed electronics and sensors to give ideas for manufacturing new BR sensors and improving the electronics for more precise acquisition of the photoelectric response in electrical point of view. This is done to increase the understanding of the photoelectric responses so it could be used in research of molecular computing [10, 19] done in the department of Information Technology in Lappeenranta University of Technology.

1.3 Structure of the Thesis

This Master's thesis continues research done earlier in Lappeenranta University of Technology by focusing on the modeling of an electrical equivalent circuit of dry BR sensor. The electrical equivalent circuit for the BR sensor is introduced based on the literature and the hypotheses. The proposed electrical equivalent circuit is then tested using measurements and simulations

Section 1 presents the background of the dry BR sensor used in this thesis and how it differs from the ones used in literature. Inspiration behind this thesis along with the objectives and restrictions of the thesis are also explained.

In Section 2, the photoelectric responses and models for the BR sensors are presented based on literature. A more detailed survey to the electrical equivalent circuits of BR is done in this section along with the reported critiques and support concerning the presented models.

In Section 3, loading effects of the measurement instrumentation in the case of opencircuit and short-circuit measurements are explained. Based on the loading effects and the literature the electrical equivalent circuit for the dry BR sensor and the equations to calculate the component values of the electrical equivalent circuit are presented.

In Section 4, measurement instrumentation for dry BR sensor is introduced and its parameters are verified. After the verification of the measurement instrumentation, photovoltage and photocurrent measurements are done to test the theory represented in Section 3. The photovoltage measurements are done by using two different light sources to measure different time constants. The measurements are done in both cases when light is introduced to BR sensor and when the light is turned off.

In Section 5, discussion about the results and the future work is reviewed. This is done to give ideas and observations for the future research of molecular computing based on the experiments gained during the work for this thesis.

Section 6 collects the results to a compact conclusion of the thesis.

2 PHOTOELECTRIC RESPONSES AND MODELS FOR BACTERIORHODOPSIN SENSOR

During illumination, BR goes through a sequence of chemical reactions which affect the wavelength of maximum absorption. This is also called the photocycle of BR and the photointermediates of the photocycle are denoted by letters, such as J, K, L, M, N and O. Each of these intermediates has different absorption maximum and lifetime. In the case of the dry BR sensor which is used in this thesis, the photocycle reduces to just photointermediates K, L and M. This is due to the dehydration of the BR sample. [16] It has also been reported [22] that no protein transfer takes place across the protein because intermediates coupling conformational change are prevented in dry BR sensors. Photocycle of the dry BR sensor can be seen from Fig. 1.



Figure 1. Photocycle of dry BR with lifetimes of the photointermediates. K, L and M are the intermediates of the photocycle and bR_{570} is the ground state (reproduced from [16]).

Photocycle of the BR sensors in suspension has more intermediates compared to the dry BR sensor but they are not presented in this thesis. Also in suspension BR sensor acts as a light-driven proton pump, but according to Wang this is not the case with dry BR sensors. [16]

2.1 Photoelectric Responses of Bacteriorhodopsin Sensor

Light-induced charge transfer in biomembranes has been referred as a photoelectric response which results in light-induced chemical reactions causing charge transfers from intermediate to other. The photoelectric response caused by the charge transfer can be seen in the measured charge, photovoltage and photocurrent responses by using appropriate measurement instrumentation. Based on this, the lifetime of each intermediate which varies from less than a picosecond to milliseconds, could be seen in the photoelectric response. [17] The photoelectric response has been firstly reported by Drachev [23], and later more components of photoelectric response have been reported [17].

Electric potential in BR charged by the light intensity, starts to decrease due to the thermal relaxation or finite resistance. This decrease can be seen as a time constant in the photoelectric response. The time constants have been marked in literature by different symbols. The time constants which are used in this thesis, are shown in Fig. 2. The responses are based on experimental results found in [5, 15, 18, 19, 24, 25, 26]



Figure 2. Shape of the input light pulse to BR sensor and the time constants used according to the electric equivalent circuit of BR. Input light pulse (a), the time constants of photovoltage response (b) and the time constants of photocurrent response (c).

Symbol PW in Fig. 2 presents the pulsewidth of the input light pulse. Symbols τ_{C1} and τ_{C2} are used to mark the time constants in the measured photocurrent responses. Consistently, symbols τ_{V1} , τ_{V2} and τ_{V3} are used to mark the time constants in the measured photovoltage responses. The photocurrent and the photovoltage responses are not in the same time scale, but the pulsewidth of the input light pulse, PW, can be used as a reference between the responses.

2.2 Electrical Equivalent Circuits of Bacteriorhodopsin

Modeling of BR has been done based on exponential fitting of the photoelectric response [5], estimating the induced charges on the electrodes of BR sensor [27] or analyzing electrical equivalent circuits modeling the BR sensor [15]. In method of exponential fitting, done also in Lappeenranta University of Technology, the measured photoelectric signal is fitted as many exponentials as the fitting process permits [10]. Displacement current method, which is also referred as estimation of the induced changes, is based on calculating the charges induced by the light using electric potential and electric field on instantaneous position. Displacement current method is used to predict the instantaneous photocurrent and photovoltage of proton transfer in an artificial BR membrane [10]. The third method, to which this thesis will be focusing on, is modeling BR using the electrical equivalent circuit.

The electrical equivalent circuit of BR has been under study over 30 years. One of the first electrical equivalent circuit of BR still is use was presented on year 1974 by Hong [15]. Hong explained that the electrical equivalent circuit of BR can be separated to two different branches: photochemical branch and inert supporting structure, membrane, formed by the PM. These parts are in parallel to each other because it has been explained that in series connection the measured voltage over capacitor $C_{\rm M}$ would be in opposite direction to what has been measured experimentally [17].

The membrane consists of parallel connection of membrane resistance, R_M and membrane capacitance C_M . The membrane of the BR has been modeled similarly in number of studies [5, 14, 16, 8]. The membrane part of the electrical equivalent circuit has been modeled, for example, the same way by Keszthelyi [25] and Walczak [8]. The difference between these two research groups is that Keszthelyi came to this conclusion by explaining that the induced charges moving towards the electrodes cause voltage between the electrodes. From photoelectric measurements they observed that the measured voltage decays exponentially like in the case of a capacitor. Because the measured BR membranes were in suspension which was conducting, he came to conclusion that the DC photocurrent had a route parallel to the BR modeled as the capacitor. For this reason, he added a resistor in parallel to the capacitor to describe the resistance countered by the DC photocurrent in suspension. [25]

Walczak [8] characterized a dry BR sensor using LCR measurements. Based on the measurements, he reported that the parallel capacitance and resistance dominate the impedance of the BR sensor so BR should be modeled as a capacitor and a resistor in parallel. Also according to Hong [17], impedance of the membrane could be measured using impedance analyzer. This is because the membrane itself is inert so it does not interact with the measurement instrumentation. Based on this, the membrane resistance $R_{\rm M}$ and capacitance $C_{\rm M}$ can be measured using impedance-measurements.

According to Hong [17], the other part of the electrical equivalent circuit of BR is the photochemical branch of BR. This part of the electrical equivalent circuit responsible of producing the photoelectric response is reported to model the photochemical event caused by illumination [15]. It is known that when light is introduced to BR, absorbed photons start the photocycle [28] which is shown in Fig. 1. During the photocycle, the charges move causing a photocurrent which can be modeled in the electrical equivalent circuit as a current source. Resistance parallel to the photocurrent source is reported to arise from the resistance from intracellular side to extracellular side of molecule [24, 17]. Due to this, the source generating the photoelectric response can be modeled as a current source with parallel resistance which can be changed, to a series voltage source and a series resistor, $R_{\rm P}$, according to Thévenin's theorem [16]. From this Hong [15] proposed a electrical equivalent circuit shown in Fig. 3.



Figure 3. Electrical equivalent circuit introduced by Hong (reproduced from [15]).

Measurement on BR sensors have revealed that the photoelectric responses are derivatives of the light introduced to it [12]. This has been explained to be caused from the fact that the voltage source generating the photoelectric response is connected to the output of BR sensor through high-pass filter [15]. For this reason the capacitor, C_P has been suggested to be in series with the voltage source, V_S , which produces the photoelectric response. The fact that the faster photoelectric responses have larger amplitude than the slower ones based on measurements supports the existence of high pass filter [17]. The series capacitance, C_P , is named, by Hong [15], as a chemical capacitance which has raised a debate between two research groups [15, 29]. According to Hong [15], the chemical capacitance is series connection of two geometric capacitances and two doublelayer capacitances which was not accepted by Trissl [29]. Trissl did not question accuracy of Hong's measurements because experimental and kinetic data reported by Hong and Mauzarall were consistent with each other. Trissl critiqued the concept of the chemical capacitance because of three reasons:

1. Hong's predicted and calculated chemical capacitance differed from each other by the order of 10^3 .

2. The system analysis is insufficiently determinate.

3. Hong did not take into account that the membrane was only partially illuminated in his circuit analysis. [29]

Trissl explained that the capacitance reported by Hong is caused by partial illumination of BR. The illuminated part of the capacitance of BR is photoactive while the nonilluminated part is unchanged [29]. Later Hong [17] responded to this critique but the concept of chemical capacitance is still not generally accepted. Hong and Trissl did not argue about the existence of series capacitance, only the formation of it. The existence of series capacitance was initially reported in year 1974 by Drachev [23] and after that by other researches [15, 16], which supports the fact that the series capacitance, C_P , exists in equivalent circuit.

Researchers [15, 16] have added resistance $R_{\rm S}$ parallel to the series capacitance $C_{\rm P}$. The resistance $R_{\rm S}$ describes DC resistance of the part generating the photoelectric response when light is introduced to the sensor [17]. This resistance differs from the membrane resistance so that the membrane resistance can be measured any time, but the resistance $R_{\rm S}$ can be measure only when light is introduced to it. This is because the photochemical branch is reported to disappear if no illumination is focused on BR [17]. Resistance $R_{\rm S}$ would change the DC-resistance measured from the BR sensor during illumination. This has been experimentally confirmed by Walczak [8] which supports the electrical equivalent circuit presented by Hong.

Also an electrical equivalent circuit which differs from the one used by Hong and Wang has been introduced by Xu [14]. Xu has the membrane impedance of BR and also the voltage source, V_s , and chemical capacitance in the electrical equivalent circuit, but they are in series. This is in conflict with the mostly used parallel connection. Xu [14] explained

the series connection by the literature [30] which defines the sign of electrogenicity, which means a change in the electrical potential of a cell, is positive when the change in dipole moment is equivalent to a shift of positive charge in the proton translocation direction of the membrane. Xu's model differs from Hong's [15] model also by the lack of series resistance R_P which formed the high-pass filter with the capacitance C_P . In Xu's model, the high-pass filter is formed by, for example, the chemical capacitance C_{ch} and the membrane resistance R_M , but also other RC high-pass filters can form. DC-resistance of the BR sensor is defined by resistor R_H as can be seen in Fig. 4. [14]



Figure 4. Electrical equivalent circuit and its simplified form introduced by Xu (reproduced from [14]).

Xu used the electrical equivalent circuit to model the behavior of BR in a monolithically integrated bio-photoreceiver. Because BR is integrated to semiconductors the design of the electrical equivalent circuit is more complicated. This makes the comparison of Hong's and Xu's models difficult. Xu has compared experimentally measured data to simulated one, but because of the difference between them and the lack of similar reported findings, no undisputed conclusion can be made. Although for simulation, Xu simplified the equivalent circuit shown in Fig. 4 to simple high-pass filter which might be the cause of the difference between the simulated and the measured photoelectric responses.

2.3 Methods to Determine the Values of Electrical Equivalent Circuit

The membrane resistance, R_M , and capacitance, C_M , of BR can be electrically measured to produce the parameter values for the electrical equivalent circuit of the membrane. This has been done using LCR-meter or impedance analyzer [8, 14]. Keszthelyi [25] has not measured the capacitance of the membrane but approximated it by using knowledge about biological membranes. Cole [31] has shown that the approximation of the membrane capacitance can be calculated based on the area of the sensor when area of 1 cm^2 corresponds to 1 μ F of capacitance and if the thickness of the sample is around 10 nm.

On the other hand, the part of BR generating the photoelectric response, also known as the photochemical branch of the electrical equivalent circuit of BR, is difficult to measure using an impedance analyzer. This is because impedance analyzer measures the impedance by using a frequency sweep. During the frequency sweep, the measured sensor should be in a steady state, but in the case of BR, the steady state measurements give just the properties of the membrane. If the light intensity is changed, the impedance of BR changes, but just for a moment so the frequency sweep has to be significantly faster than the response from BR to accurately measure the impedance.

Fastest response of BR has been reported by Xu [7] to be 1.68 picoseconds. Xu has measured the fastest reported risetime of 1.68 ps using electro-optic sampling where the laser pulse with a pulsewidth of 500 fs was introduced to the dry BR film. Using this measurement, Xu was able to achieve a resolution of better than 1 ps. [7] Using electronics, measured responses have not been as fast as with the electro-optic sampling because the loading of the BR sensor. The measured responses have been usually in the range of microseconds [17] so for the analyzer depending on a frequency sweep, this is usually impossible. It could be possible to measure the BR sensor's impedance at higher frequencies by measuring one frequency at the time. Then the measurement time is limited just by the period of the measured frequency. Even this cannot give information about the frequencies which period is longer or about the same than the time constant of the measure response. For this reason, analysis of the electrical equivalent circuit is done using the measured photoelectric responses and the time constants.

In literature, measurements have been done using three methods. The first one is the short-circuit method also called a voltage clamp method where the measurements are done under short-circuit conditions. Measurement instrumentation in this method has an input impedance of zero so by using this method, the photocurrent from the BR sensor can be measured. The second method is the open-circuit or a current-clamp method where the measurements are done by connecting the measurement instrumentation with high input impedance to the BR sensor to prevent current from flowing through the measurement instrumentation. Using this method the voltage over the sensor can be measured.

The third method is the debated [29] tunable voltage clamp method. In this method, the input impedance of the measurement instrumentation is taken into account in the electrical equivalent circuit and its varied to optimize the measurement of BR's membrane. According to Hong [15] the advantage of this is that the use of the voltage clamp method

provides only one time constant in case of BR, but the tunable voltage clamp method enables more complete measurement and description of the membrane. Another advantage of the method is that the deviation from the voltage clamp method in terms of error is meaningless [15]. The method has not been used or confirmed by anyone else so the method could be questioned. For this reason the tunable voltage clamp method is not used in this thesis.

3 ELECTRICAL EQUIVALENT CIRCUIT FOR DRY BACTERIORHODOPSIN SENSOR

Bacteriorhodopsin sensor is known to be a capacitive high-impedance source which is confirmed by our previous research [19, 10] and literature [17, 5]. For this reason there has been several reports [5, 18, 16, 12] about how does the measurement instrumentation affect the electrical responses from the sensor. To measure a high-impedance source like a BR sensor, the loading of the measurement instrumentation must be taken into account. Before analyzing the effects of loading, electrical equivalent circuit for the whole system must be designed.

The membrane of BR has been the most studied part of the electrical equivalent circuit of BR and it has been generally accepted to model the membrane using a resistance R_M and a capacitance C_M in parallel [15, 25, 5, 16, 8]. The part of the electrical equivalent circuit responsible for the photoelectric response has been modeled differently by Xu and Hong as explained in Chapter 2.2. The membrane and the part responsible for generating the photoelectric response was selected to be in parallel to each other. The fact that the impedance changes caused by the light have been reported [8], supports the parallel connection of the inert membrane and the part generating the photoelectric response. In parallel connection, the total DC resistance decreases during illumination because the membrane resistance R_M and the resistance R_S of the part generating the photoelectric response are in parallel as reported in [8]. Also the fact that other researcher except for Xu, have been using parallel connection supports this hypothesis.

There has been reports [17, 18] that the measured photoelectric responses are derivatives of the light intensity and this has been explained to be due to the high-pass filter between the voltage source, V_S , and the output of the BR sensor. Also the existence of the capacitance in series with the the voltage source, V_S has been reported by more than one researcher [15, 16] and it has not been questioned. Resistance R_S shown in Fig. 3 on page 15 has been used by Wang [16] and Hong [15] also, but its necessity has not been reported.

Because the necessity of resistor R_S has not been reported, the part responsible for generating the photoelectric response was chosen to be modeled, in this thesis, only by a RC high-pass filter without the resistor R_S . Even thought the Walczak's report supports the existence of resistor R_S , its effect on the total resistance has been less than 1 % of the total DC resistance [8]. The membrane was modeled similarly with the literature. Based on this, the proposed electrical equivalent circuit of BR is as shown in Fig. 5.



Figure 5. Electrical equivalent circuit of BR designed in this thesis.

Also the fact that the voltage response in Fig. 2c on page 13 resembles impulse response of the high-pass filter without the oscillations, even though the input is an square pulse, supports the selected 1st order electrical equivalent circuit. The reason why the photovoltage response resembles impulse response of high-pass filter is because the input light pulse is only 10 μ s compared to the length of photovoltage responses which are around millisecond based on literature [17]. Similar result can be seen from the photocurrent response which also resembles output of a high-pass filter in Fig. 2b.

Because it has been determined that the measurement instrumentation affects the measurements, the electrical responses do not describe just the BR sensor but a system which consist of BR sensor and measurement instrumentation [17, 12, 18, 5]. The measuring of the BR sensors photoelectric responses is done by using voltage amplifier [15], transimpedance amplifier (TIA) [14] or charge amplifier [11]. Amplifiers differ from each other by the physical quantity they are amplifying. In this study, focus is on voltage- and transimpedance amplifiers because this enables us to measure both the photocurrent and the photovoltage from the dry BR sensor. The voltage amplifier has ideally infinite input resistance, but in reality it is difficult to find an operational amplifier with input impedance over hundreds of teraohms.

The measurement instrumentation has been modeled by a resistor in majority of the studies [15, 14, 16]. Usually the reported impedance of the measurement instrumentation consists of a input resistance and a input capacitance. The measurement instrumentation is modeled using only a resistance because it affects the measurements more than the input capacitance which is usually few picofarads in the case of the measurement instrumentation. Resistance, on the contrary, can chance from kiloomhs to hundreds of teraohms depending on whether the selected device is optimized to measure high-impedance sources or not. In this research, the input capacitance of the measurement instrumentation is taken into account along with the input resistance because it increases the accuracy of the model. The input capacitance has been said by Keszthelyi [25] to affect the photoelectric response which supports the necessity of the input capacitance in the electrical equivalent circuit of BR.

It has been also reported that the capacitance of the BR sensor decreases if the sample thickness is increased [8] as can be seen from Fig. 6.



Figure 6. Membrane capacitance of BR sensor divided by area versus average thickness (reproduced from [8]).

Now if the sensor is made thick and its area is decreased, the capacitance of BR sensor decreases. If the BR sensor is made thick and it has small area, the capacitance of the BR sensor might be equal to the input capacitance of the measurement instrumentation. Now if the input capacitance of the measurement instrumentation is ignored, the error to the measurements might be significant. So to model the behavior of BR sensors of all sizes accurately, the input capacitance of the measurement instrumentation should be modeled in the electrical equivalent circuit.

In this thesis, the resistance of the contacts connecting the measurement instrumentation to BR sensor has been ignored because the value of contact resistance is less than 1 milliohm. This is calculated from the resistance of the contacts which is about $90 * 10^{-9}$ Ω and total length of the contacts is 4 cm. Full electrical equivalent circuit to model the measurements of BR can be seen in Fig. 7.



Figure 7. The proposed electrical equivalent circuit of the system formed by a BR sensor and the measurement instrumentation.

3.1 Loading Effect of High-impedance Measurement Instrumentation

Light intensity causes the electric charges to move and charge BR's membrane capacitance modeled by capacitor $C_{\rm M}$. This causes voltage, $U_{\rm ph}$, over the capacitor. If the measurement instrumentation's input capacitance, $C_{\rm IN}$, is taken into account, the electric charges charge the total capacitance, $C_{\rm tot}$, formed by the parallel connection of the membrane capacitance, $C_{\rm M}$, of the BR sensor and the input capacitance, $C_{\rm IN}$, of the measurement instrumentation. Finite input resistance, $R_{\rm IN}$, of the amplifier and the membrane resistance, $R_{\rm M}$, of the BR sensor cause a leakage current to flow through these resistors $R_{\rm IN}$ and $R_{\rm M}$ causing discharge in the capacitors, $C_{\rm M}$ and $C_{\rm IN}$. During measurements, this discharge of voltage $U_{\rm ph}$ can be seen as time constant $\tau_{\rm V3}$ which is defined in Fig. 2 on page 13.

When light is switched off, because of the leakage current, the voltage, $U_{\rm ph}$, at the capacitors $C_{\rm M}$ and $C_{\rm IN}$ starts to decrease by the rate defined by the parallel connection of membrane resistance $R_{\rm M}$ of the BR sensor and the input resistance $R_{\rm IN}$ of the measurement instrumentation. So by increasing the input impedance of the measurement instrumentation the voltage $U_{\rm ph}$ over the capacitors $C_{\rm M}$ and $C_{\rm IN}$ will decrease slower. The same has been mathematically explained without the input capacitance $C_{\rm IN}$ of the measurement instrumentation by Hong [17]. Because ignoring the input capacitance $C_{\rm IN}$ might cause an error to the measurement, the input capacitance $C_{\rm IN}$ was taken into account. By adding the input capacitance $C_{\rm IN}$ in parallel to the membrane capacitance $C_{\rm M}$, the modified equation is as follows:

$$\tau_{\rm V3} = (C_{\rm M} + C_{\rm IN}) \frac{R_{\rm M} * R_{\rm IN}}{R_{\rm M} + R_{\rm IN}},\tag{1}$$

From the Eq. (1) can be also seen that by increasing the input capacitance, C_{IN} , of the amplifier, the discharge of the voltage, U_{ph} , slows down. This is because the same amount of charges do not charge a larger capacitance to as high voltage as a smaller capacitance. This relationship can be expressed as follows:

$$U_{\rm ph} = \frac{Q_{\rm ph}}{C_{\rm tot}},\tag{2}$$

where Q_{ph} is the charge generated in BR by the light. If the capacitance C_{tot} increases because of the measurement instrumentation's input capacitance C_{IN} , the measured photovoltage U_{ph} decreases. Smaller photovoltage U_{ph} decreases the leakage current through the measurement instrumentation and the membrane of BR slowing down the discharge rate and lengthening the time constant τ_{V3} . This discharge caused by the finite input resistance R_{IN} of the measurement instrumentation might not be understood and the photoelectric response received from the measurement instrumentation might be wrongly analyzed and thought to be, for example, a response caused by a transition in BR's photocycle.

Eq. (1) has been experimentally tested by Wang [16]. If values reported by Wang: $\tau_{V3} = 2.5 \text{ s}$, $R_{IN} = 10 \text{ G}\Omega$, $R_M = 650 \text{ G}\Omega$, $C_M = 63 \text{ pF}$, $C_{IN} = 0 \text{ pF}$ are inserted to Eq. (1) the calculated time constant, τ_{V3} , do not respond to the reported value [16]. The calculated time constant, τ_{V3} , would be about 620 ms. From the same equation, Wang [16] has calculated the time constant, τ_{V3} , to be 2.5 s which differs significantly from the value calculated by us. Inconsistency between the given values and the reported result of the calculation raises question concerning the reliability of the Eq. (1) even though its used by two research groups [17, 16].

Charge rate of the BR sensor is limited by a low-pass filter which forms in the electrical equivalent circuit of BR. This low-pass filter forms from the internal resistance $R_{\rm P}$ of the voltage source generating the photoelectric response and the series connection of series capacitance $C_{\rm P}$ and the total capacitance $C_{\rm tot}$. This low-pass filter can be seen in the measured photovoltage $U_{\rm ph}$ as the time constant $\tau_{\rm V1}$ defined in Fig. 2 on page 13. Equation to calculate the time constant $\tau_{\rm V1}$ has been introduced by Wang [16], but he has not taken the input capacitance $C_{\rm IN}$ of the measurement instrumentation into account. Because the input capacitance $C_{\rm IN}$ is in parallel to the membrane capacitance $C_{\rm M}$, it increases the total capacitance $C_{\rm tot}$ lengthening the time constant $\tau_{\rm V1}$. By adding the input capacitance, $C_{\rm IN}$

to Wangs equation modified definition for the time constant, τ_{V1} , is as follows:

$$\tau_{V1} = R_{\rm P} \frac{C_{\rm P} * (C_{\rm M} + C_{\rm IN})}{C_{\rm P} + C_{\rm M} + C_{\rm IN}}$$
(3)

Eq. (3) has been reported only by Wang [16], but it has been experimentally tested in the same paper just like Eq. (1). If the Eq. (3) is solved using the given values above along with two others: $C_P = 63$ pF and $R_P = 16$ G Ω , the calculated time constant, τ_{V1} , would be 0.5 s which differs from the time constant, τ_{V1} , 2 s reported by Wang [16]. Another notable finding is that the reported and calculated time constants τ_{V1} and τ_{V3} both differ from each other by the factor 4 in the cases of Eq. (1) and Eq. (3). So by dividing the reported time constants using factor 4, the measured and the calculated time constants would be consistent. The fact that the both time constants differ from the time constants τ_{V1} and τ_{V3} might be wrong because of an error while writing the paper, not because of incorrect hypotheses.

3.2 Short-circuit Measurement

The transimpedance amplifier, TIA, has been used to measure the photocurrent of BR [8]. The input impedance of the TIA is ideally zero. In reality it is easier to implement than the required high input impedance of the voltage amplifier compared to impedance of BR. This is because the impedance of the dry BR sensor is in gigaohms. When using a transimpedance amplifier the input impedance is low so it can be noted that the current through BR is insignificant compared to the current through TIA. So the current through BR can be ignored without making a significant error. This error caused by the impedance of BR causes larger error when voltage amplifier is used because the difference between the impedance of BR and the input impedance of amplifier is smaller than in the case of TIA. Also because TIA has a zero input impedance it does not affect the measurement of BR.

The loading caused by the input impedance of the voltage amplifier does not realize with the transimpedance amplifier. This is because the non-inverting pin of the amplifier is grounded and the inverting pin is in virtual ground of the amplifier. In short, transimpedance amplifier enables us to measure the sensor using a short circuit method. In this method, the membrane impedance of BR which consists of the membrane resistance $R_{\rm M}$ and the membrane capacitance $C_{\rm M}$, can be ignored because it is parallel to TIA. Now what is left is the part generating the photoelectric response of the equivalent circuit as shown in Fig. 8.



Figure 8. The electric equivalent circuit of BR when TIA amplifier circuit is used.

Now the transfer function of the electrical equivalent circuit in the case of TIA can be determined. The result can be expressed as follows:

$$\frac{I_{\rm ph}}{V_{\rm S}}(s) = \frac{1}{R_{\rm P}} \frac{R_{\rm P} C_{\rm P} s}{1 + R_{\rm P} C_{\rm P} s} \tag{4}$$

From the transfer function, the transconductance $1/R_{\rm P}$ and the time constant $\tau = R_{\rm P}C_{\rm P}$ of the high-pass filter can be found.

4 MEASUREMENTS AND RESULTS

4.1 Measurement Instrumentation

All measurements were done using an unoriented dry BR sensor. Precise description of the preparation of the dry BR sensor used in our test setup can be found in [10] and [19]. The dry BR sensor was measured to determine the value of BR's membrane capacitance $C_{\rm M}$. The measurements were done using the impedance analyzer and a detailed description can be found in [10]. The measurements gave the membrane capacitance, $C_{\rm M}$, an average value of about 100 pF. The measurements were done in room temperature and without light present.

The measurement set up used in this thesis consists of: light source, BR sensor, measurement instrumentation and oscilloscope which are shown in Fig. 9.



Light source

Figure 9. Measurement set up used in the measurements. The light source was varied between the light step measurement of the photovoltage and the two other measurements. The measurement instrumentation was varied in the photovoltage measurements.

The oscilloscope used in all of the measurements during this thesis was Agilent infiniium DSO812904A with bandwidth of 12 GHz. The light source of the photocurrent measurement and the light pulse measurement of the photovoltage was Cavitar Cavilux SMART laser. The laser emission wavelength was 690 nm and the pulsewidth of a single light pulse could be chosen from 30 ns to 10 μ s [32]. During the measurements the light source was set to 10 μ s pulsewidth and to the maximum power of 400 W.

In light step measurement of the photovoltage, the light source was Green 10 W LED with typical wavelength of 523 nm. Wavelength of the LED was selected to be close to the maximum absorption of BR [10]. Change of the light source was done to enable use of light step. For the diode laser, the maximum length of the light pulse was 10 microseconds which is not long enough to measure the time constant τ_{V3} which has been reported [17, 18, 12] to be milliseconds long. Using the LED with a suitable driver, the maximum pulsewidth is limited only by the heating of the LED. The LED was driven

using a PP400 LED Lighting Controller. Current through the LED was set to 5 % of the rated current so that the measurement instrumentation would not saturate and the LED would not overheat.

Because it was essential to have a light pulse with a fast risetime in the photocurrent measurement and the light pulse measurement of photovoltage, the risetime of the laser needed to be measured. The risetime must be faster than the measured dry BR sensor so it would be possible to say that the light source does not affect the measurement of the rising edge of the BR sensor. For this reason, the rising edge and the pulsewidth of the laser source was measured. Light detection was done using a Centronic OSD15-E - photodiode. The photodiode's current signal was amplified and converted to voltage using a transimpedance amplifier.

The TIA was selected as a photodiode amplifier over voltage amplifier because its zero input impedance. The zero input impedance causes that the photodiode capacitance does not affect the bandwidth of the TIA like in the case of voltage amplifier. For this reason, it has a improved bandwidth compared to voltage amplifier. Also current monitoring offers better linearity than voltage monitoring because TIA removes, for example, the voltage swing over the diode which is one of the causes of nonlinearity. [33]

Transimpedance gain, $R_{\rm F}$, of the photodiode amplifier was set to 160. The gain was chosen to be as large as possible to increase the signal to noise ratio (SNR) of the circuit without clipping the response received from the photodiode. SNR of the TIA amplifier is increased when the gain is increased because the gain increases the desired signal directly but the signal noise according to $\sqrt[2]{R_{\rm F}}$. Increase in the transimpedance gain also decreases the bandwidth of the amplifier making the transimpedance gain selection an optimization between SNR, gain and bandwidth.[33] The measured power of the laser diode light source using the photodiode circuit and the designed circuit are shown in Fig. 10.

From Fig. 10a, it can be seen that the laser pulsewidth is about the same as the nominal value given by the manufacturer [32]. The risetime of the light pulse is limited by the TIA because it has been informed by the manufacturer [34] that the photodiode itself has a rise time of 12 ns and capacitance of about 100 pF. It is true that the TIA isolates the diode capacitance from the output of the amplifier but it is only a valid assumption in lower frequencies. When the frequency is increased, the diode capacitance and the input capacitance of the amplifier set the upper frequency pole that limits the bandwidth of the amplifier by causing peaking in the frequency response of the amplifier configuration. Peaking can be suppressed by using a feedback capacitor $C_{\rm F}$. An optimal value of $C_{\rm F}$



Figure 10. Normalized power of the laser diode light source with the source set to 10 μ s pulsewidth and maximum power (a). The simplified schematic of transimpedance photodiode amplifier circuit used in the simulation (b).

flattens the frequency response giving the maximum flat bandwidth of the amplifier. [33] The value of the feedback capacitance, seen in Fig. 10b, was selected according to simulations done using Cadence OrCAD Capture 16.0 software. The value of capacitance used in simulations was 0.5 pF larger than the value in Fig. 10b because a printed circuit board (PCB) layout can easily add 0.5 pF parasitic capacitance to the feedback loop of the circuit [33].

The transimpedance amplifier for the dry BR sensor was designed in our previous research and the design procedure is explained in [20]. The transimpedance amplifier's performance was also tested to measure the bandwidth of the amplifier. If the photocurrent measured from BR is slower than the maximum risetime of the amplifier, it can be noted that the current is generated by the BR sensor and the amplifier does not filter the measured photocurrent. Testing of the performance was done by simulation using the Cadence OrCAD Capture 16.0 software. The amplifiers were modeled using spice models given by the manufacturers. The circuit and circuit parameters used in the simulation are shown and explained in Appendix A. The simulated rising edge of the amplifier is shown in Fig. 11

The simulation results in Fig. 11 shows that the rise time of the TIA is slightly over 100 ns. This is fast enough because in previous research the fastest time constants measured from BR have been microseconds long [19].

Also different voltage amplifiers with varying input characteristics and gains have been designed in the previous research [21, 19]. This was done because by varying the in-



Figure 11. Simulated output voltage of the TIA used to amplify the photocurrent measured from the BR sensor.

put impedance of the measurement instrumentation, the time constant τ_{V3} should vary according to theory presented in Section 3. To increase the amount of different voltage amplifiers, capacitors of 22 pF, with a tolerance of $\pm 5 \%$ [35] were added to amplifier VA4 and VA6. Voltage amplifiers VA1-VA4 have the same circuit, but the input characteristics are varied. Also all of the voltage amplifiers' have a gain of 10 expect for the voltage amplifier VA2 which has a gain of 100. Also the circuits of voltage amplifiers' VA5 and VA6 are the same, but amplifier, VA6, has added input capacitance compared to amplifier VA5. To confirm that the voltage amplifiers' characteristics are what they were designed to be, the voltage amplifiers' performance and input characteristics were measured. The input resistance was measured using Fluke 187 True RMS multimeter. The type A uncertaincy of measurement, which varied between different resistance ranges, was determined from the manufactures manual [36]. The measurement results and uncertainties are shown in table 1.

Table 1. Measured and nominal input characteristics of voltage amplifiers

	Nominal	Nominal	Measured	Measured
Amplifier	resistance	capacitance	resistance	capacitance
	$\mathbf{M}\Omega$	pF	$\mathbf{M}\Omega$	pF
VA1 G10	2	6	2.01 ± 0.0007	8±0.5
VA2 G100	2	6	$2.00{\pm}0.0007$	$8{\pm}0.5$
VA3	44	6	44.3 ± 1.529	$8{\pm}0.5$
VA4	44	28	44.3 ± 1.529	28 ± 1
VA5	220	2.5	217±21.9	$2.3{\pm}0.2$
VA6	220	24	217±21.9	25 ± 1

The values of the input capacitances were measured using a HP 4194A Impedance Analyzer. The values of the input capacitance, C_{IN} , were function of frequency. Detailed explanation and plotted results can be seen in Appendix B. From table 1, it can be seen that the measured capacitance values differ from the nominal values just few picofarads. This small difference between the nominal and measured values confirms the fact that the amplifiers input capacitances are what they were designed to be. This small increase in the input capacitance values can be result from the parasitic capacitance coming from the PCB layout. The parasitic capacitance could be easily more than 0.5 pF if it has not be taken into account while designing the PCB [33]. The input resistance, R_{IN} , is also about the same as given, but the uncertainty of resistance measurements increases at the higher resistance values because of the multimeter. For this reason, the largest values of resistances can be measured with the tolerance of 10 %. If these values are used in equations, they cause an minimum error of 10 % to the results of calculation.

Also the bandwidths of the voltage amplifiers' were determined to confirm that the amplifiers do not affect the photovoltage measured from the BR sensor. The bandwidths were determined using a step response. Test signal with a risetime of 1 nanosecond, peak-topeak voltage of 10 millivolts and length of 20 milliseconds was generated by using HP 8130A pulse generator. The test signal was measured because it defines the fastest bandwidth that could be measured by using the pulse generator. The measured risetime of the test signal is shown in Fig. 12. The length of the pulse was also measured to be the set value of 20 milliseconds.



Figure 12. The measured risetime of the test signal generated by using HP 8130A pulse generator.

As can be seen from the Fig. 12, the peak-to-peak voltage is 10 mV and risetime of the test signal is about 1 ns. At the rising edge of the test signal, there is some damping oscillation for the first 6-7 ns and the amplitude of the oscillation is about 10 % of the amplitude of the test signal. The oscillation lasts just few nanoseconds and it's amplitude is small compared to the whole step so it can be said that it does not affect the measurements. Now that the test signal is measured, it can be used to measure the step responses of the voltage amplifiers. The normalized results are shown in Fig. 13



Figure 13. The measured and normalized step responses of the voltage amplifiers using the test signal generated by HP 8130A pulse generator

The amplifiers' VA5 and VA6 had the the fastest measured risetimes which were about 20 ns. From the Fig. 13, the instrumentation amplifiers' VA1, VA3 and VA4 risetimes were about the same, 320 ns, which was expected because the amplifier circuits and the gains were the same. The amplifier VA4 has the added input capacitance which seems to increase the risetime slightly, compared to amplifiers VA1 and VA3 which have no added input capacitance. VA2's gain of 100 decreases the bandwidth and the risetime of the circuit and for that reason it has the slowest risetime. The risetime was about 850 ns which still is faster than the measured risetimes from the BR sensor which were microseconds [19]. From this can be confirmed that the amplifiers do not affect the measured risetimes from the BR sensor if they are slower than 850 nanoseconds.

4.2 Light Pulse Measurement of Photovoltage

The objective of the light pulse measurement of the photovoltage was to verify if the time constant τ_{V1} could be calculated using Eq. (3). By changing the input impedance of the measurement instrumentation, it should be seen that the input resistance of the amplifier has no effect on the time constant τ_{V1} and the input capacitance affects the time constant according to equation (3). Time constant τ_{V1} of the BR's voltage response was measured and the results were normalized to make the comparison of the responses easier. The results are shown in Fig. 14.



Figure 14. Measured and normalized time constants τ_{V1} of the voltage amplifiers when light is introduced to BR.

Measurements were done only when light was switched on because the limited pulsewidth of the laser made the switch-off measurement impossible. The measurements were done using voltage amplifiers VA1-VA3 and VA5-VA6. Despite the fact that not all of the amplifier were not used, the measurement is still reliable because the range of different amplifiers and parameters the measurements can be though to be inclusive.

The rising edge of the responses lasted 10 microseconds which was the length of the light pulse. So with 10 microsecond pulsewidth the time constant τ_{V1} cannot be directly seen from the response but using exponential fitting the fast time constant can be approximated from the response. Even though the time constant cannot be precisely seen from the Fig. 14, a good approximation of it could be estimated. The voltage in Fig. 14 is close to reaching the steady state so a conclusion can be drawn that the time constant is somewhere

around 4-6 μ s without making significant error. Another notable results which can be seen from Fig. 14 is that the time constant of the voltage amplifiers is about the same. This confirms that the input resistance of the measured amplifiers which vary from 2 M Ω to 220 M Ω seem not to have an effect on the rising edge of the measured responses. The measurement of the input resistance, R_{IN} , can be though to be reliable because the input resistance, R_{IN} , of the measurement instrumentation was varied in the range of over two decades.

Also changing the input capacitance of the measurement instrumentation from 6 pF to 28 pF does not seen to have an effect on the measured responses. In the measurement of the input capacitance, C_{IN} , was varied in a narrower range compared to the input resistance. This caused the expected time constant changes to be smaller compared to the expected changes caused by the input resistance of the measurement instrumentation. From Eq. (3), it can be seen that the input capacitance increase should change the time constant maximum of 550 ns which corresponds to about 15 % increase in the time constant. So because the expected change in the time constant τ_{V1} is only 15 % and the measured photovoltage do not reach the steady state because of the limited pulsewidth of the light source, it cannot be confirmed that the input capacitance has not effect on the time constant τ_{V1} .

4.3 Light Step Measurement of Photovoltage

Objective of the second measurement was to confirm whether the time constant τ_{V3} changes with the input impedance of the measurement instrumentation. Also to confirm if the time constant τ_{V3} behaves according to equation (1). It has been reported [16] that the capacitance of BR changes during illumination. This reported change in capacitance of BR should affect the time constant τ_{V3} so the measurements were done in both cases when light was switched on and when it was switched off to confirm the reported change in capacitance. The measurement results were normalized. The time constants of the voltage amplifier when light is introduced to BR are shown in Fig. 15

From Fig. 15, it can be seen that the photovoltage decays faster when the measurements are done by using voltage amplifiers VA1 and VA2 than by using voltage amplifiers VA4-6 which have larger input impedance. Faster decay means also that the time constant τ_{V3} decreases. Changing the input resistance seem to affect the time constant τ_{V3} so clearly that its effect to the time constant can be confirmed. Also the difference in the time constants, τ_{V3} , of the measured photovoltage between amplifiers VA3 and VA5, which do



Figure 15. Measured and normalized time constants τ_{V3} of the voltage amplifiers when light was turned on.

not have added capacitance, and amplifiers VA4 and VA6, which input capacitance is increased, is significant. This confirms that the input capacitance, C_{IN} , of the measurement instrumentation affects the decay rate of the measured photovoltage.

It can be also noted that the changing of the input capacitance of the measurement instrumentation affects the time constant τ_{V3} more between the amplifiers VA3 and VA4 than with the amplifiers VA5 and VA6. What makes these results significant is that the effect of the measurement instrumentation's input capacitance on the photoelectric response has been reported by Keszthelyi [25] but no experimental results confirming the issue has not been reported until now.

The photoelectric responses were also measured when light was turned off. These results are shown in Fig. 16. Comparing figures 15 and 16, it can be seen that the photovoltage decays slower when the light is switched off. Also it seems that the change in the decay rate between the switch-on and switch-off is larger in the amplifiers with lower input impedance. In amplifiers VA5 and VA6 the change between whether light is turned on or off is almost nonexistent. This measurement confirmed that the capacitance of the dry BR sensors change when the illumination is changed as reported by Wang [16].

The effects of the measurement instrumentation's input capacitance and input resistance seen from Fig. 15 can be also seen in Fig. 16. This further supports the fact that input impedance of the measurement instrumentation has an effect on time constant τ_{V3} . By



Figure 16. Measured and normalized step responses of the voltage amplifiers when light was turned off.

increasing the input impedance of the measurement instrumentation the time constant τ_{V3} increases.

4.4 Light Pulse Measurement of Photocurrent

Photocurrent from the dry BR sensor was measured by using the designed transimpedance amplifier [20] to verify the proposed electrical equivalent circuit shown in Fig. 11 on page 30. The measured photocurrent is shown in Fig. 17.

From the measured photocurrent, the time constant τ_{C2} was measured to be 20.6 μ s. To verify the hypothesis about the proposed electrical equivalent circuit, the component values for the electrical equivalent circuit, shown in Fig. 8, were calculated using equations (3) and (4). The calculated values were $R_P = 43 \text{ k}\Omega$ and $C_P = 470 \text{ pF}$. Output of the electrical equivalent circuit was simulated using SIMULINK software and then added to the Fig. 17 (yellow line). As it can be seen from Fig. 17, the simulated response corresponds to part of the measured photocurrent, but the time constant τ_{C1} of the measured photocurrent response is less than the simulated one when light is on. This could be caused by the fact that the intrinsic properties of the dry BR sensor change with the illumination as reported [16] and confirmed by our measurements above.

It has been reported that the time constant, when BR is illuminated, is about half of the



Figure 17. The measured photocurrent of the dry BR sensor and the simulated high-pass filter responses. Yellow line is the response from the high-pass filter when the component values are calculated by using the time constant τ_{C2} . Green line is the response from the high-pass filter when the component values are calculated by using the time constant τ_{C1}

time constant when light is turned off [16]. Because the values of the C_P and R_P where calculated using the time constant τ_{C2} found when light is switched off, the calculated values match the response only when the light is switched off. When BR is illuminated, it is possible to calculate the values for the equivalent circuit matching the response when light is on by using the time constant τ_{C1} , which is about 6 μ s. If resistance R_P is kept the same, the value of C_P can be calculated to be 139.5 pF. To verify whether this is true, the response was simulated and the result is shown in Fig. 17. As seen from Fig. 17, the calculated value using time constant τ_{C1} (green line) match the measured response when BR is illuminated.

From this it can be concluded that the hypothesis about the electrical equivalent circuit shown in Fig. 8 on page 26 is a good approximation to model the behavior of the photocurrent response measured from dry BR sensor. The component values of the electrical equivalent circuit change depending the illumination. This further supports the results produced by using the voltage amplifiers that the dry BR sensor should be modeled as time-variant system.

In literature it was reported [8] that the photocurrent is a derivative of the induced charges. To confirm this the measured photocurrent was integrated in SIMULINK software. The SIMULINK model consisted of an integrator-block into which the photocurrent was fed and from the output of the integrator-block, the integrated response could be received. The

simulated response was then compared to the measured response of the voltage amplifier VA5. The integrated photocurrent and the measure photovoltage response of amplifier VA5 are shown in Fig. 18.



Figure 18. The measured photovoltage response of amplifier VA5 and the integrated photocurrent response.

The rising edge of the integrated current is about the same shape as the rising edge of the measured voltage response. This confirms that the rising edge of the voltage responses is the result of the electrical equivalent circuit shown in Fig. 8 and the membrane impedance of BR or the measurement instrumentation do not affect it. Also according to this the input capacitance seems not to have an effect on the time constant τ_{V1} .

It has been reported [12] that the measured photovoltage over the total capacitance C_{tot} is directly related to the induced charges as can be seen from Eq. (2). So by examining the shape of the integrated response from TIA and by comparing it to the response received from voltage amplifier VA5 in Fig. 18. It can be seen that the photocurrent measured by using transimpedance amplifier from the dry BR sensor is in fact a derivate of the voltage response caused by the light pulse as reported by Takamatsu [12].

5 DISCUSSION

5.1 Results

Effect of the input resistance and capacitance on the time constant τ_{V3} was confirmed in this thesis. From the results, it can be reported that the measurement instrumentation should be modeled by using both the input resistance, R_{IN} , and the input capacitance, C_{IN} , like was done in this thesis. Based on the literature [8, 17, 16, 25] the membrane of BR was modeled by using the membrane resistance R_M and the membrane capacitance C_M in parallel. Part of the electrical equivalent circuit shown in Fig. 19, which generates the photoelectric response, was proposed to form from the resistance R_P and series capacitance C_P . This was confirmed by the photocurrent measurements. Also values for the resistance R_P and the capacitance C_P were calculated using Eq. (4) introduced in this thesis.



Figure 19. The proposed electric equivalent circuit of the system formed by a BR sensor and the measurement instrumentation.

The measured change in time constant τ_{V3} between the cases when light was turned on and off was 30 % which differed from the 50 % change reported by Wang [16]. This difference might be caused by the fact that the prepared dry BR sensors are unique and the light intensity used in the measurements also affects the impedance of BR along with the time constant τ_{V3} as reported by Walczak [8]. This measured change in photovoltage's time constant, τ_{V3} , between switch-on and switch-off confirmed the time-variant behavior of BR. The time-variant behavior of the photocurrent could be explained by changing the component values in the proposed electrical equivalent circuit. Also a method to calculate the values in both switch-on and switch-off cases was presented. During our measurement it was noticed that the change in the time constant τ_{V3} between switch-on and switch-off decreases when input resistance of the measurement instrumentation increases. This might be a reason why the time-variant behavior of BR has not been noticed until now by anyone else than Wang because the measurements of the photovoltage has been done in literature [12, 16] by using amplifiers with input resistances' of gigaohms and teraohms.

In the light step measurement of the photovoltage, it was noticed that the change in time constant τ_{V3} caused by the added input capacitance of the measurement instrumentation, is greater between the amplifiers VA3 and VA4 than between the amplifiers VA5 and VA6. This is probably because the input impedance increases more in the case of the amplifier VA4 than with the amplifier VA6, when 22 pF capacitor was added to the input of the amplifier. The measured photovoltage responses lasted seconds so they were at the low frequencies and at the low frequencies the input resistance, R_{IN} , plays a bigger part in the input impedance of the measurement instrumentation. For this reason the input capacitance C_{IN} affects the time constant τ_{V3} more with amplifiers with smaller input resistance.

The Eq. (1) introduced by Hong which should explain the photoelectric response caused by the loading of the measuring instrumentation, was experimentally tested by Wang [16]. In this thesis, it was noticed that the experimental results reported by Wang had inconsistency between the reported values and the equations used to calculate them. This raised the question concerning the reliability of the reported experimental results. To test Eq. (1) introduced by Hong [17], the membrane resistance $R_{\rm M}$ of BR was calculated by using the values measured in this thesis. Equation, where the membrane resistance is solved, is as follows:

$$R_{\rm M} = \frac{-R_{\rm IN}\tau_{\rm V3}}{\tau_{\rm V3} - (C_{\rm IN} + C_{\rm M})R_{\rm IN}}$$
(5)

The values used in Eq. (5) were $C_{\rm M} = 100$ pF, $C_{\rm IN} = 2.3$ pF, $R_{\rm IN} = 220$ M Ω and $\tau_{\rm V3} = 24.6$ s. The result of the calculation was a negative value for the membrane resistance. This confirms that Eq. (1) cannot be used to calculate the component values in electrical equivalent circuit.

5.2 Future Work

Before the effect of the measurement instrumentation's input capacitance to the time constant τ_{V1} can be confirmed, new measurements should be done to make sure that the input capacitance do not follow the modified Eq. (3). In the new measurements, the difference between the input capacitance of the measurement instrumentation and the membrane capacitance of BR should be minimized then it could be certainly stated whether the input capacitance has any effect on the time constant τ_{V1} . This would be possible if new BR sensors with lower capacitance would be manufactured, then the input capacitance of the measurement instrumentation should have a greater effect on the time constant τ_{V1} . Also if the measurements could be done by using a light step, the effect of the input capacitance could be seen more easily because the photovoltage would reach the steady state. The measurement of the time constant τ_{V1} using the light step would need a light source with the risetime of the laser and the pulsewidth of the used LED driver.

The time constant τ_{V3} could not be calculated by using Eq. (1) on page 24. To explain the photoelectric response which is affected by the loading of the measurement instrumentation, a transfer function was lead from Fig. 5 on page 21. The transfer function is shown in simple form in Eq. (6) and constants A-D are explained in Eq. (7) - (10).

$$H_{S,full} = \frac{As}{Bs^2 + Cs + D} \tag{6}$$

$$A = R_{\rm IN} R_{\rm M} C_{\rm P} \tag{7}$$

$$B = (C_{\rm IN} + C_{\rm M})C_{\rm P}R_{\rm IN}R_{\rm M}R_{\rm S}$$
(8)

$$C = R_{\rm IN}R_{\rm M}C_{\rm IN} + R_{\rm IN}R_{\rm M}C_{\rm M} + R_{\rm IN}R_{\rm M}C_{\rm P} + R_{\rm M}R_{\rm S}C_{\rm P} + R_{\rm IN}R_{\rm S}C_{\rm P}$$
(9)

$$D = R_{\rm IN} + R_{\rm M} \tag{10}$$

In the future, the transfer function in Eq. (6) should be modified to a form which shows the time constants of the system. Using the time constants, values needed for the electrical equivalent circuit of BR and a new equation replacing Eq. (1) could be solved.

Knowledge concerning the measurement instrumentation and the dry BR sensor's electrical characteristics has increased during the research. For this reason matters has been noticed that should be processed before continuing the research. One of these matters is the BR sensor. New dry BR sensors could be prepared to ease the measurements and to increase its performance by decreasing the capacitance of the BR sensor. For example, if the dry BR sensor is used as an fast receiver, its thickness should be increased. This is because BR functions as a capacitor and if capacitors dielectric thickness is increased, its capacitance decreases due to the fact that:

$$C = \epsilon \frac{A}{d},\tag{11}$$

where C is the capacitance of the capacitor modeling the dry BR sensor, A is the area of the dry BR sensor's electrodes and d is the thickness of BR between the electrodes. The same has been suggested by Wang [16] and experimental observations [17, 8] concerning the issue support this assumption. So in the future if new BR sensors will be made they should be thicker as explained. From Eq. (11), it can be seen that the area of dry BR sensors electrodes is directly proportional to the capacitance of the sensor. So the sensors area should also be smaller to produce faster BR sensors. According to literature, the electrical orientation of the samples does not seem to have effect on the capacitance of BR [8]. So by orienting the BR samples, the capacitance of the BR sensor stays the same, but it has been reported [8] that using orientation the measured amplitude of the photoelectric response can be increased to hundred times greater than what it is without orientation. Orienting the samples using an electric field, the photoelectric response can be strengthened which is because of the vectorial nature of the proton translocation within BR [8].

Also the measurement instrumentation could be redesigned according to the new information received from the measurements. Using the transimpedance amplifier, photoelectric responses can be measured with minimal interaction to the sensor. Problem with the designed transimpedance amplifier was its poor SNR. SNR could be increased by increasing the transimpedance gain with the feedback resistor R_F , but this decreases the bandwidth of the TIA. For this reason, changing the operational amplifier to another faster one enables increase of gain and SNR with the same bandwidth. Bandwidth cannot be increased to gigahertzs with needed gain due to the gain-bandwidth product of operational amplifiers which is few gigahertzs even with the fastest operational amplifiers.

One possible way of designing faster TIA, is to used radio frequency (RF) transistors. Using, for example, MMG3007NT1 RF transistors which have a bandwidth from DC to 6 GHz, it is possible to connect them in cascade to increase the gain of the circuit without decreasing the bandwidth. The same kind of TIA amplifiers can be found in literature [37, 38, 39], but some of them are manufactured directly to silicon without the use of ICs. Nonetheless, use of discrete transistors might be a possibility, if faster TIA is needed.

6 CONCLUSION

The photoelectric measurements and the measurement instrumentation for a BR sensors have been under intensive study. Different equivalent circuits have been presented in literature [15, 7, 8], but they cannot explain the photoelectric responses accurately when the measurement instrumentation is connected to the BR sensor. For this reason, further research concerning the photoelectric measurements needs to be done. In this Master's thesis, the objective was to explain the photoelectric responses measured from the dry BR sensor. This was done by using the proposed electrical equivalent circuit for the dry BR sensor and the measurement instrumentation.

In this study, the proposed electrical equivalent circuit for the dry BR sensor was presented and experimentally tested. The proposed electrical equivalent circuit modeled the behavior of the photocurrent by using less components that the electrical equivalent circuits found in literature [7, 15, 16]. The simulated photocurrent responses and the measured ones corresponded to each other which confirms the proposed electrical equivalent circuit valid.

The proposed electrical equivalent circuit modeling the photovoltage response measured from the dry BR sensor was modified from the ones presented in literature [15, 16]. Also the equations introduced [17] to explain the loading of the measurement instrumentation have been experimentally tested by Wang [16], but the reported experimental results had inconsistent calculations. Also the calculations gave a negative value for the resistance if the component values and the measured time constants reported in this thesis were used. For this reason, a question concerning the reliability of the equation was raised.

Also the relationship between the integrated photocurrent and photovoltage response reported by Walczak [8] was experimentally tested and confirmed in this thesis. This conclusion is based on the observation that the integrated photocurrent corresponded to the measured photovoltage. The integrated photocurrent's time constant on the rising edge of the response was reported to be almost equal to the time constant τ_{V1} measured from the dry BR sensor by using the voltage amplifiers. This confirmed that the time constant τ_{V1} of the photovoltage response was not affected by the membrane impedance of the dry BR sensor. If the membrane impedance of BR would affect the photovoltage response, the time constant τ_{V1} and the integrated photocurrent would not be equal.

One of the objectives of this thesis was to confirm the change in the capacitance of the dry BR sensor reported by Wang [16]. The capacitance was measured, in this thesis, in

both the photocurrent and the photovoltage measurements, to change with the illumination. It was also noticed that the change in the capacitance of the dry BR sensor decreases between the switch-on and switch-off if the input impedance of the measurement instrumentation is increased. This might be the reason why these kind of results have not been published until now by anyone else than Wang [16]. Based on this, the dry BR sensor was confirmed to be a time-variant system.

References

- M. Halseman and S. Hauck. The future of integrated circuits: A survey of nanoelectronics. *Proceeding of the IEEE*, 98(1):12–38, 2010.
- [2] N. Hampp, C. Bruchle, and D. Oesterhelt. Bacteriorhodopsin and its variants as media for dynamic holographic information processing. In *Third International Conference on Holographic Systems, Components and Applications*, pages 35–39, 1991.
- [3] D. Oesterhelt and W. Stoeckenius. Rhodopsin-like protein from the purple membrane of halobacterium halobium. *Nature new biology*, 233(39):149–152, 1971.
- [4] M. C. Petty, M. R. Bryce, and D. Bloor. *Introduction to molecular electronics*. Hodder Headline PLCl, 1995.
- [5] H. W. Trissl. Photoelectric measurements purple membranes. *Photochemistry and Photobiology*, 51(6):793–818, 1990.
- [6] P. Silfsten, S. Parkkinen, J. Luostarinen, A. Khodonov, T. Jääskeläinen, and J. Parkkinen. Color-sensitive biosensors for imaging. In *Proceedings of ICPR '96*, volume 3, pages 331–335, 1996.
- [7] J. Xu, A. B. Stickrath, P. Bhattacharya, J. Nees, G. Vàrò, J. R. Hillebrecht, L. Ren, and R. R. Birge. Direct measurement of the photoelectric response time of bacteriorhodopsin via electro-optic sampling. *Biophysical Journal*, 85(2):1128–1134, 2003.
- [8] K. A. Walczak. Electronic characteristics of bacteriorhodopsin. In *8th IEEE Conference on Nanotechnology*, pages 553–556, 2008.
- [9] G. Vàrò and L. Keszthelyi. Photoelectric signals from dried oriented purple membrane of halobacterium halobium. *Biophysical Journal*, 43(1):47–51, 1983.
- [10] L. Lensu. *Photoelectric properities of bacteriorhodopsin films for photosensing and information processing*. PhD thesis, Lappeenranta University of Technology, 2002.
- [11] S. Takamatsu, K.i Hoshino, K.i Matsumoto, T. Miyasaka, and I. Shimiyama. Biomolecular image sensor of bacteriorhodopsin patterned by electrodeposition. In 18th IEEE International Conference on Micro Electro Mechanical Systems, pages 847– 850, 2005.
- [12] S. Takamatsu, K.i Hoshino, K.i Matsumoto, T. Miyasaka, and I. Shimiyama. The photo charge of a bacteriorhodopsin electrochemical cells measured by a charge amplifier. *Electronics Express*, 8(7):505–511, 2011.

- [13] V. Renugopalakrishnan, S. Khizroev, L. Lindvold, P. Li, and H. Anand. Proteinbased memory. In *International Conference on Nanoscience and Nanotechnology*, pages 228–230, 2006.
- [14] J. Xu, P. Bhattacharya, and G. Vàrò. Monolithically integrated bacteriorhodopsin semiconductor opto-electronic integrated circuit for a bio-photoreceiver. *Biosensors* and Bioelectronics, 19(8):885–892, 2004.
- [15] F. T. Hong and D. Mauzerall. Interfacial photoreactions and chemical capacitance in lipid bilayers. *Proc. Nat. Acad. Sci. USA*, 71(4):1564–1568, 1974.
- [16] W. W. Wang, G. K. Knopf, and A. S. Bassi. Photoelectric properities of a detector based on dried bacteriorhodopsin film. *Biosensors and Bioelectronics*, 21(7):1309– 1319, 2006.
- [17] F. T. Hong. Interfacial photochemistry of retinal proteins. *Progress in Surface Science*, 62(1-6):1–237, 1999.
- [18] B. Yao, Y. Wang, M. Lei, and Y. Zheng. Characteristics and mechanisms of the two types of photoelectric differential response of bacteriorhodopsin-based photocell. *Biosensors and Bioelectronics*, 19(4):283–287, 2003.
- [19] T. Tukiainen. Photoelectric measurements and modeling of bacteriorhodopsin. Master's thesis, Lappeenranta University of Technology, 2008.
- [20] J. Talvitie. Bakteerirodopsiini-sensorin puskurointi transimpedanssivahvistimella ja toteutuksen suunnittelu, Bachelor's thesis. Lappeenranta University of Technology, 2010.
- [21] J. Toikka. Bakteerirodopsiini-sensorin jännitevahvistus ja toteutuksen suunnittelu, Bachelor's thesis. Lappeenranta University of Technology, 2010.
- [22] C. Ganea, C. Gergely, K. Ludmann, and G. Vàrò. The role of water in the extracellular half channel of the bacteriorhodopsin. *Biophysical Journal*, 73(5):2718–2725, 1997.
- [23] L. A. Drachev, A. D. Kaulen, S. A. Ostroumov, and V. P. Skulachev. Electrogenesis by bacteriorhodopsin incorporated in a planar phospholipid membrane. *FEBS Letters*, 39(1):43–45, 1974.
- [24] B. E. Fuller, T. L. Okajima, and F. T. Hong. Analysis of the d.c. photoelectric signal from model bacteriorhodopsin membranes: d.c. photoconductivity determination by the null current method and the effect of proton ionophores. *Bioelectrochemistry and Bioenergetics*, 37(2):109–124, 1995.

- [25] L. Keszthelyi and P. Ormos. Displacement current on purple membrane fragments oriented in a suspension. *Biophysical Chemistry*, 18(4):397–405, 1983.
- [26] K. Bryl, G. Vàrò, and R. Drabent. The photocycle of bacteriorhodopsin immobilized in poly(vinyl alcohol) film. *FEBS Letters*, 285(1):66–70, 1991.
- [27] L. Keszthelyi and P. Ormos. Electric signals associated with the photocycle of bacteriorhodopsin. *FEBS Letters*, 109(2):189–193, 1980.
- [28] A. Popp, M. Wolperdinger, N. Hampp, C. Bräuchle, and D. Oesterhelt. Photochemical conversion of the o-intermediate to 9-cis-retinal-containing products in bacteriorhodopsin films. *Biophysical Journal*, 65(4):1449–1459, 1993.
- [29] H. W. Trissl. The concept of chemical capacitance: A critique. *Biophysical Journal*, 33(2):233–242, 1981.
- [30] C. Gergely, C. Ganea, G. Groma, and G. Vàrò. Study of the photocycle and charge motions of the bacteriorhodopsin mutant d96n. *Biophysical Journal*, 65(6):2478– 2483, 1993.
- [31] K. S. Cole. *Membranes, ions, and impulses: a chapter of classical biophysics.* University of California Press, 1968.
- [32] Cavitar. CAVILUX R Smart Laser light for monitoring and [retrieved & D,[year unknown] October 24, 2011]. From: http://www.cavitar.com/liitetiedostot/editori_materiaali/45.pdf.
- [33] J. Graeme. Photodiode amplifiers: OP AMP solutions. McGraw-Hill, 1995.
- [34] Centronic. Centronic silicon photodetectors series E datasheet, [year unknown] [retrieved November 18, 2011]. From: http://www.farnell.com/datasheets/311218.pdf.
- [35] Taiyo Yuden Co.,LTD. *Electrical characteristics LMK107BJ474K [product info]*, [year unknown] [retrieved November 18, 2011].
- [36] Fluke Corporation. *Fluke model 187 and 189 true RMS multimeter users manual*, 2000 [retrieved November 19, 2011]. From: http://assets.fluke.com/manuals/187_189_umeng0200.pdf.
- [37] M. J. N. Sibley and R. T. Unwin. Transimpedance optical preamplifier having a common-collector front end. *Electronics Letters*, 18(23):985 – 986, 1982.
- [38] J. Martinez-Castillo and J. Silva-Martinez. Transimpedance amplifiers for optical fiber systems based on common-base transistors. In *Proceedings of the 1999 IEEE International Symposium on Circuits and Systems*, volume 6, pages 85 – 88, 1999.

- [39] Y. Lu and M. N. El-Gamal. A 2.3 v low noise, low power, 10 ghz bandwidth sibipolar transimpedance preamplifier for optical receiver front-ends. In *The 2001 IEEE International Symposium on Circuits and Systems*, volume 4, pages 834 – 837, 2001.
- [40] Texas Instruments. Wideband, unity-gain stable, JFET-input operation amplifier OPA659 datasheet, 2008 [retrieved November 18, 2011]. From: http://www.ti.com/lit/ds/symlink/opa659.pdf.
- [41] Hewlett Packard. Impedance/Gain-Phase analyzer HP 4194A manual, 1996 [retrieved November 19, 2011]. From: http://cp.literature.agilent.com/litweb/pdf/04194-90011.pdf.
- [42] Agilent Technologies. Agilent 16047D Test Fixture operation and service manual, Third edition, 2000 [retrieved November 19, 2011]. From: http://cp.literature.agilent.com/litweb/pdf/16047-90300.pdf.

Appendix A. Simulation Model Selection and Analysis

The simulation model was designed according to the amplifier circuit used in the measurements. The dry BR sensor was modeled using only a current source to examine the properties of the amplifier circuit: if the measured response would be slower than the amplifier, it could be that the response is caused by the dry BR sensor. The feedback resistance and the gain, were experimentally chosen to values as large as possible to increase the SNR of the circuit without decreasing the bandwidth of the TIA so much that it would affect the measurement results. Feedback capacitance was chosen to a slightly larger value than based on simulation would be sufficient. This was done so it would be certain that no peaking would be present in the frequency response. In the previous impedance measurements [10], BR's capacitance have been measured to be from 80 pF to 130 pF so it is possible that the capacitance is larger than the 100 pF which was used in the simulations.

If the capacitance of the BR sensor would be greater than the value used in the simulations, a larger feedback capacitance would be needed to flatten the frequency response. The chosen value of the feedback capacitance to the measurements was 3 pF. This value is about the same as the value given in the manufacturer's datasheet which supports the fact that the selection of the feedback capacitance is correct. In the reality, the feedback capacitance is greater because the PCB adds parasitic capacitance to the feedback loop of the TIA. Parasitic capacitance is usually approximately 0.5 pF even if its effects has been minimized while designing PCB [33]. For this reason, the simulation was done by using feedback capacitance of 3.5 pF. The simulation circuit used in the simulations is shown in Fig. AA.1.



Figure AA.1. Simulation model for the transimpedance amplifier used in the photocurrent measurements.

The voltage amplifier of the TIA circuit had a gain-bandwidth product (GBWP) of 1.5 GHz so it does not affect the bandwidth of the circuit. This is because, with the gain of

Appendix A. (continued)

110, the bandwidth is still over 13 MHz. OPA659's bandwidth can be calculated using equation (12) given by the manufacturer [40].

$$f_{-3\mathrm{dB}} = \sqrt{\frac{GBWP}{2\pi R_{\mathrm{F}}C_{\mathrm{F}}}},\tag{12}$$

where f_{-3dB} is the upper -3 dB cut-off frequency, GBWP is the gain-bandwidth product, $R_{\rm F}$ is the feedback resistor and $C_{\rm F}$ is the feedback capacitance. From the equation can be seen that the bandwidth of OPA659 is about 6 MHz which is less than half compared to LMH6624. So it can be reported that OPA659 is limiting the bandwidth of the TIA circuit. OPA659's bandwidth could be increased by decreasing the transimpedance gain and increasing the voltage gain, but it would decrease the SNR of the output.

Appendix B. Measurement Results of Voltage Amplifiers' Input Capacitance

The voltage amplifiers' input capacitances were measured by using HP 4194A Impedance analyzer. The input capacitances are functions of frequency so no exact value can be calculated. The input capacitance used in the calculations must be chosen so that it describes the input capacitance as well as possible, in the frequency range of the measurements. The measurement instrumentation limited the frequency range from 100 Hz to 40 MHz when HP 16047D Test Fixture was used [41, 42], but the use of HP 41941A Impedance Probe improved the bandwidth of the measurement instrumentation from 10 Hz to 100 MHz [41]. During the measurements, it was noticed that HP 4194A + HP 16047D Test Fixture cannot measure the input capacitance when the measured frequency was less than 6.3 kHz and the same effect could be seen when frequency was about 40 MHz. This is probably due to the impedance analyzer which cannot measure the impedance accurately in the neither ends of the instrumentation's frequency range even though it should according to the manufacturer [41].

The results using the impedance probe were not better because the frequency range was better only on higher frequencies. Also the impedance probe was sensitive to movement which became a problem because the lack of contacts what could be connected to tightly to the BR sensor. HP 4194A + HP 16047D Test Fixture was selected instead of the impedance probe because the voltage amplifiers had bandwidths of only few megahertz so the capacitance values at the frequencies over 10 MHz do not effect the measurement. Also the HP 16047D Test Fixture enabled the measured amplifier to stay still without a connection to a measurer. With no connection to the measurer, he does not affect the measurement by his' own capacitance, increasing the reliability of the measurement. For this reason, the measured values were plotted on Fig. AB.1 only from 10 kHz to 30 MHz. The measurements were done using the following settings: medium integration time and averaging of 4 samples.

The input capacitance in Fig. AB.1 do not vary significantly within the frequency range from 10 kHz to 1 MHz, but after that the capacitance starts to vary in the case of VA1-VA4. This is probably because of the characteristic of INA111 instrumentation amplifier which was used in the voltage amplifiers VA1-VA4. INA111 does not have as wide bandwidth as OPA659 and LMH6624 which were used in the voltage amplifiers VA5-VA6. This is probably because the input characteristics are not designed to such high frequencies. It seems that the added input capacitance in VA4 causes the capacitance to decrease at the lower frequency than the voltage amplifiers VA1-VA3. The circuits in the voltage amplifiers VA1-VA4 are exactly the same, so the reason for the faster decrease in the input capacitance is caused by the added capacitor. This is confirmed by the capacitor's electrical characteristics in Fig. AB.2 where can be seen that the resonance point of the

Appendix B. (continued)



Figure AB.1. Input capacitances of the voltage amplifiers as a function of frequency. The voltage amplifier VA1 is in two different circuits. One used in BR measurements and the other without the input resistors to show that the lack of input resistors do not affect the input capacitance of the amplifier.

impedance is in few megahertz, after that the capacitors starts to act like an inductor [35].

Because of the voltage amplifier's bandwidth, this decrease in capacitance does not affect our measurements and the input capacitances used in the calculation can be selected from the measurements. Another notable observation was that using the Impedance Analyzer HP 4194A the input capacitance of the voltage amplifiers VA1-VA4 could be measured only when one of the input pins of the amplifier was connected to the power supply ground. The voltage amplifiers VA1-VA4 have paths for bias currents from input pins to ground using two equal size resistors, R_{IN} , which define the input resistance of the amplifier circuit. Grounding the other input pin changes the input resistance, but this makes no difference because only the input capacitance was measured. The only possible error for the input capacitance measurements comes from the lack of parasitic capacitance of the removed resistor R_{IN} . To confirm if the parasitic capacitance of the resistor R_{IN} changes the input capacitance significantly, the voltage amplifier VA1 was also measured so that the both of the R_{IN} resistors were removed. Without the input resistors R_{IN} , the only capacitance affecting the circuit is amplifiers' input capacitance and parasitic capacitance of the PCB, which do not change if the resistor R_{IN} is or is not used.

From Fig. AB.1, it can be seen that in the case where the resistor R_{IN} is not used, the input capacitance differs from the input capacitances of the voltage amplifiers VA1-VA3 about

Appendix B. (continued)



Figure AB.2. Impedance and ESR of the capacitor used to add input capacitance to the amplifier VA4 (reproduced from [35]).

one picofarad in range of 10 kHz to 30 MHz. The input capacitance in the voltage amplifiers VA1 to VA3 should all have the same value of input capacitance, but in the measurements it differs also about one picofarad. From this can be concluded that the removing of the resistor $R_{\rm IN}$ do not affect the capacitance measurements and the values describing the input capacitance, as well as possible, in the frequency range of amplifiers can be selected. Selected values are shown in table AB.1. Uncertaincy of the measurements was selected based on the estimation how accurately the capacitance selection could be made from Fig. AB.1.

	Measured
Amplifier	capacitance
	pF
VA1 G10	8 ± 0.5
VA2 G100	8 ± 0.5
VA3	8 ± 0.5
VA4	28 ± 1
VA5	2.3 ± 0.2
VA6	25 ± 1

Table AB.1. Measured and given input characteristics of voltage amplifiers

The type A uncertaincy of the measurements was not calculated because it was a function of a frequency just like the measured capacitance.