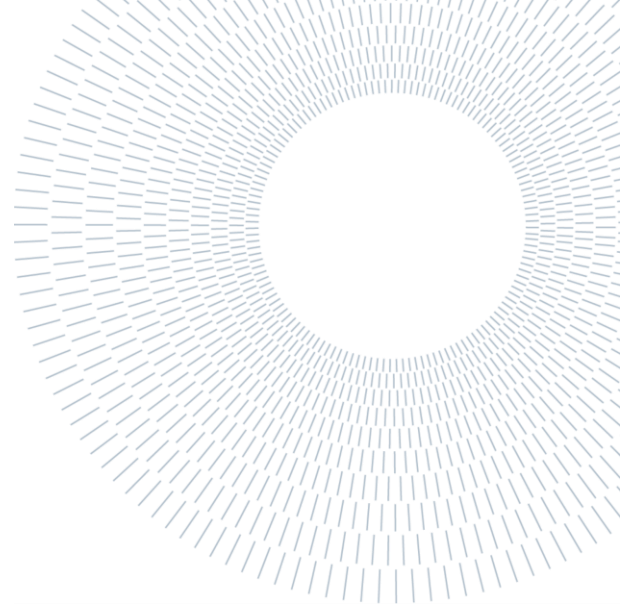




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EXECUTIVE SUMMARY OF THE THESIS

Enhancing electrophysiological signal recording: innovations in optrode design and technology compared to standard measurement instrumentation

TESI MAGISTRALE IN BIOMEDICAL ENGINEERING – INGEGNERIA BIOMEDICA

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ACADEMIC YEAR: 2022-2023

1. Introduction

The field of electrophysiology (EP) plays a crucial role in understanding the physiological and pathophysiological functions of excitable cells and tissues by measuring ionic currents across cell membranes, either from single cells or tissues [1]. EP signal measurement is vital for monitoring patients' health, and as technology continues to advance in the biomedical field, there is a growing need for more powerful and accurate techniques for recording EP signals. Traditionally, electrophysiology has relied on metal electrode arrays, but the introduction of optical-based technologies presents a new frontier in the field. Optical electrodes, or optrodes, offer an alternative approach by using light to sense and transmit EP signals, overcoming some limitations of traditional electrode systems by converting signals from electrical to optical domains at the tissue interface. However, further development and testing are

necessary to match the performance of current conventional electrode systems.

This thesis aims to characterize and mitigate artifact signals that still affect EP signals recorded using optrodes. These artifacts may arise from various sources, including but not limited to light sources, equipment issues, and utility frequency (50 Hz and 60 Hz), with particular emphasis on stimulation artifacts when simultaneously stimulating and recording from tissues or cells. Additionally, redesigning the optrode sensor packaging is proposed as a potential solution to improve performance, focusing on enabling connection to a wide variety of EP electrodes.

2. New OpBox design

In the following sections, when discussing the updated optrode packaging the designation OpBox will be used. The term is coined from the term "optrode" and "box" (where the transducer is housed) in order to assign a more commercially oriented name. One of the main objectives of this

project is to improve the packaging of the optrode transducer to reduce stimulation artefacts and to allow recording with different electrodes but with the use of one optrode transducer as a universal ‘headstage.’ To this end, investigations were made into how conventional headstages are constructed, with the aim of trying to reproduce something similar with optrode transducers. Another aspect that was deemed essential to address is the implementation of a multi-registration set-up package, to better eliminate artefacts. One optrode transducer will be connected to the recording electrode and its return electrode positioned at the point of interest for recording the signal, the other transducer will be connected to the same type of electrodes, but these will still be positioned away from the recording point of interest. In this way, through signal processing techniques such as subtraction, the artefact can be removed from the signal of interest. In Fig.1 is possible to visualise the final OpBox packaging. An electromagnetic shielding box (EMI) made of aluminium is used as the main container, because of its effectiveness in reducing the presence of electromagnetic interferences from the surrounding environment, and its light weight and its good conductivity. The optical connection is made on the left side of the box, which is then directly connected to the photodetector via an optical circulator, while the electrical connection is on the right side of the box, providing accessibility for plugging in various electrodes.

3. Case studies

To come up with a better design and better understand the problems most neuroscience researchers face during their experiments, several end-users were interviewed, in order to understand the various application scenarios.

The following three different studies recruited are running at UNSW in the School of Medical Sciences, Faculty of Medicine and in the School of Biomedical Engineering and are explained below in order to better understand their research areas.

1. Electrical stimulation in the nerve: the aim of this study is to characterise and model the neural effects of invasive and non-invasive nerve stimulation in human and rat peripheral nerve[2].

2. Measuring brain activity following hybrid electrical/optical stimulation of the eyes: the aim of this study is to assess simultaneous electrical and optical (hybrid) stimulation as a method to reduce energy requirements, and its potential as an artificial vision treatment [3].
3. Electrical stimulation for artificial vision: this study focuses on characterising the response of four major functionally different retinal ganglion cells (RGCs) to a high frequency stimulus (HFS) paradigm [4].

In the following paragraphs, the above three studies will be referred as: “Nerve experiment,” “Cornea ring experiment,” “Artificial vision experiment” respectively, for simplicity and compactness. For all the studies a focus on the different type of recording and stimulating electrodes that have been used is done, reported, in Table1.

Experiments set up

A series of experiments will be conducted to assess the performance of the OpBox in comparison to traditional measurement instrumentation. These experiments will involve replicating the three user scenarios discussed above. Each of them will be replicated in a saline environment. In addition, the nerve experiment, will be followed by assessments using nerve tissue post-mortem and in vivo. Within each scenario, comparisons will be drawn between unpackaged transducers and the OpBox configuration. Through these comparisons, valuable insights into the OpBox's effectiveness in minimising artefacts and improving signal fidelity across diverse experimental conditions will be gained.

Software

In all the experiments, LabChart (ADInstruments, ANZ), in conjunction with a PowerLab recording unit, is used. It offers versatile data acquisition and analysis solutions. The systems are used together with computers to record and analyse physiological signals from human and animal subjects. In one compact unit, PowerLab systems perform the functions of chart recorders, XYT plotters, digital voltmeters, and storage oscilloscopes. LabChart also supports the export of a file in MATLAB format.

For this project all the signal analysis has been done with MATLAB software. Furthermore, to properly analyse all the recorded signal, the subtraction technique, reported in Equation 1, has been used to remove the baseline shifts

4. Results

4.1. Nerve experiment

Fig. 2 reports the results obtained with the standard measurement instrumentation (Fig. 2A) compared to the one obtained with the OpBox (Fig. 2B). The similarity in artefact amplitude between the two conditions (around 4mV) suggests that both can capture similar signal intensities. However, the key advantage of the OpBox, lies in its ability to start and end the artefact noise precisely at the same time as the biphasic stimulus pulse, without any recovery time. This feature is crucial because it ensures that there is no overlap between the artefact and the physiological signal of interest which is expected to occur after the stimulus pulse, reducing the risk of data loss or distortion. By eliminating the need for recovery time, researchers can capture the signal of interest more accurately and efficiently, leading to a clearer understanding of the underlying electrophysiological processes. The absence of 50 Hz noise in the signal obtained with the OpBox compared to the evident presence of this noise in the signal from the standard measurement instrumentation is indeed another crucial finding.

Ex vivo experiment

In Fig. 3 a zoomed-in view comparison is made between the performances of the standard measurement instrumentation and the OpBox in terms of artefact response. What is particularly noteworthy in Fig. 3A, the bio-amplifier case, is the visualisation of an exponential decay. The exponential decay may distort the original signal waveform, making it difficult to accurately interpret or analyse the underlying physiological events. This distortion can affect the fidelity of the recorded data and may lead to erroneous conclusions. In contrast to the standard case, the OpBox signal (Fig. 3B) consistently demonstrates a lack of significant recovery time. The absence of a significant recovery time in the OpBox setup underscores its advantage in providing reliable

and high-quality signal recordings, enhancing the overall efficiency and effectiveness of experimental investigations. However, in the OpBox signal, there is a limited exponential rise, after the artefact stimulus, which should not impact the signal processing.

In vivo experiment

Different stimulation protocols have been used, in order to define a stimulation threshold: 0.75mA pulse amplitude (biphasic) was identified. This threshold signifies that nerve responses were detected when the stimulation pulse amplitude exceeded 0.75mA, whereas below this threshold, only artefacts were observed. The nerve response can be properly visualised from Fig. 4, where a stimulus of 1 mA (above threshold) was delivered. Despite the slightly lower peak-to-peak amplitude in the standard measurement instrumentation case compared to the OpBox case, it is crucial to observe the duration of the artefacts. In the standard measurement case (Fig. 4A), the artefacts exceed the 2 ms biphasic pulse duration, leading to uncertainty and difficulties in interpreting the nerve response. The prolonged artefacts may overlap with the nerve response, hindering a clear analysis. However, in the case of the OpBox (Fig. 4B), this peak is absent, providing clearer and more reliable data for analysis. Furthermore, Fig. 5, reaffirms the earlier observations. In this instance, no nerve response is expected to be visualised as the stimulus delivered is below the threshold. However, in the standard measurement case (Fig. 5A), an amplifier ringing is apparent. Conversely, in the OpBox case (Fig. 5B), the stimulus artefact ends precisely when the stimulus pulse concludes. Regarding the peak-to-peak difference, in the OpBox case, it is slightly lower than in the standard measurement instrumentation case. This observation further affirms its capability to maintain low artefact amplitudes.

4.2. Cornea ring experiment

Fig. 6 illustrates a zoomed-in view of the complete signal acquisition process for both the bioamplifier and OpBox. Despite employing a notch filter in the bioamplifier setup (Fig. 6A), the persistent presence of 50 Hz noise remains noticeable. In contrast, the OpBox configuration (Fig. 6B) operates effectively without the necessity for supplementary filtering

adjustments and significantly reduces the amplitude of the 50 Hz oscillations. Additionally, the OpBox configuration demonstrates a reduced artefact amplitude in comparison to the bioamplifier configuration, indicating superior performance in terms of signal fidelity and interference mitigation. These findings underscore advantages of the OpBox, which delivers enhanced noise reduction and artefact suppression without the requirement for additional filtering adjustments.

4.3. Artificial vision experiment

When replicating experiments using patch pipette recording electrodes, challenges arise due to the unique characteristics of each electrode, including variations in size, resistance, and surface properties. Manipulating these electrodes requires precision, as subtle differences in positioning can affect recording quality. Furthermore, variations in electrode placement can significantly influence the shape and characteristics of the measured artifact, leading to differences in recorded measurements. Consistent electrode positioning is essential for ensuring the repeatability and reproducibility of experimental results, as inconsistent placement can introduce variability and hinder the ability to draw valid conclusions. Therefore, maintaining consistent electrode positioning is crucial for reliable data comparison and interpretation in patch pipette recording experiments. Overall, for the reasons stated above the “Artificial vision experiment” could not be considered for an accurate comparison between the simple optrode and the OpBox, due to the difficulty of maintaining the same electrode positioning between the two different experiments set up, both with the bioamplifier, the optrode and the OpBox.

5. Equations, Tables, Figures

5.1. Equations

$$N(t) = S(t) - \mu$$

Equation 1: Subtraction technique.
N(t): normalised signal. **S(t):** original signal.
 μ : mean value of the original signal.

5.2. Tables

Studies	Recording electrode	Stimulating electrode
Nerve experiment	Monopolar hook electrode	Surface electrode
Cornea ring experiment	Multi-channel electrode array	Cornea ring electrode
Artificial vision experiment	Monopolar patch pipette	Single platinum electrode

Table 1: Recording and Stimulating electrodes for multiple user cases.

5.3. Figures

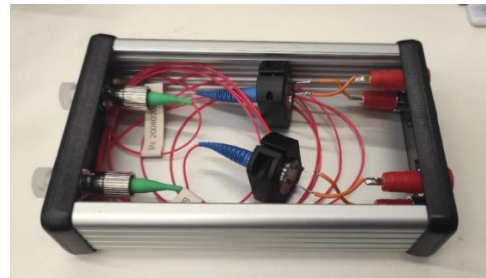


Figure 1: OpBox

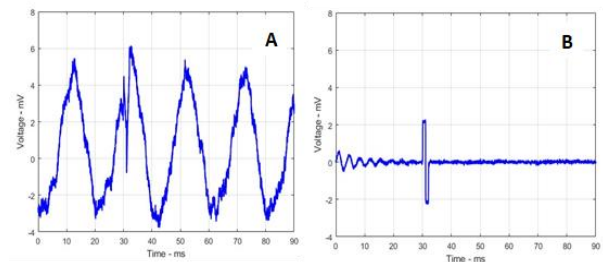


Figure 2: Nerve benchtop experiment. Stimulus artefact measurements in saline. (A) Standard measurement instrumentation. (B) OpBox. Displayed signals are an ensemble average of 100 repeats.

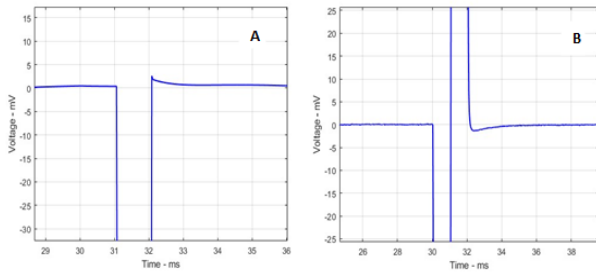


Figure 3: Zoomed- in view ex vivo nerve benchtop experiment. Stimulus artefact measurements in saline (A) Standard measurement instrumentation. (B) OpBox. Displayed signals are an ensemble average of 100 repeats.

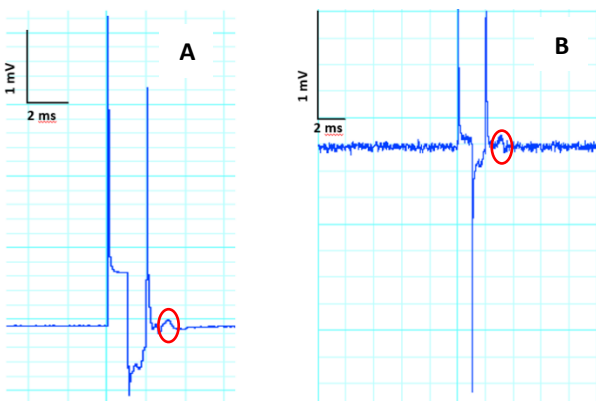


Figure 4: In vivo sciatic nerve experiment. Measured responses to a 1 mA, 1ms biphasic stimulus in a rat. (A) Standard measurement instrumentation. (B) OpBox. Displayed signals are ensemble average of 100 repeats. Nerve response highlighted in red circle

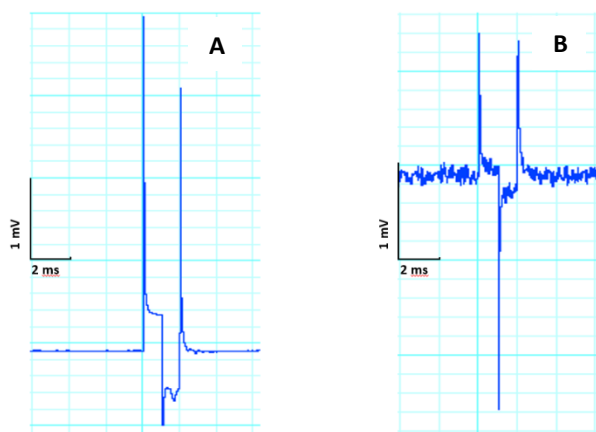


Figure 5: In vivo sciatic nerve experiment. Measured responses to a 0.5 mA, 1ms biphasic stimulus in a rat. (A) Standard measurement instrumentation. (B) OpBox. Displayed signals are ensemble average of 100 repeats.

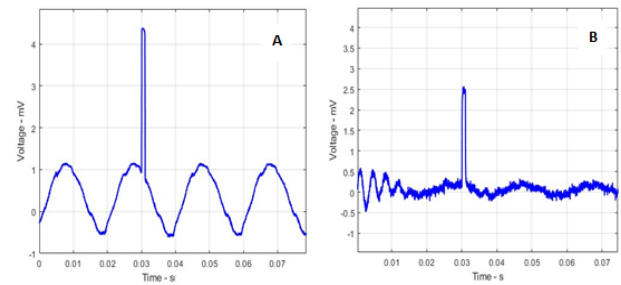


Figure 6: Zoomed- in view cornea benchtop experiment. Stimulus artefact measurements in saline (A) Standard measurement instrumentation. (B) OpBox. Displayed signals are an ensemble average of 100 repeats.

6. Conclusions

The research encompassed in this work spans for a novel optical electrode. Its working principle was discussed in detail, demonstrating the new sensor packaging design as well as the considerations needed for the success of the device. In this report has been proved the ability of one optrode transducer to be a universal ‘headstage,’ to work with electrodes of various types, together with improvements in minimising artefacts, and relative sensibility to environmental factor has been achieved. In conclusion, in this thesis, I played a central role in characterising and refining the optrode device, tackling crucial challenges like minimising stimulation artefacts and enhancing device packaging. Through extensive experimental work, I conducted thorough electrical and optical analyses of the sensor, employing benchtop assessments and refining software for precise measurements. Conducting an in vivo experiment not only enabled me to further assess the reliability of my previous findings but also paved the way for future development in the field. The implications of my results and enhancements are substantial for the ongoing advancement of optrode neurotechnology.

References

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7. Acknowledgements

I would like to thank you Prof. Fiorini, who without his approval this project could have been undertaken. Thanks for encouraging me to embark on this path, and even if from a distance, his presence was crucial for me. A special thanks to Dr. Amr and Dr. Nigel Lovell and all the GSBME research group at UNSW for warmly welcoming me into his laboratory with great enthusiasm.