Infrared Neural Modulation
Photothermal Effects on Cortex Neurons Using Infrared Laser Heating

Qingling Xia

Licentiate Thesis

Division of Neuronic Engineering
Department of Biomedical Engineering and Health Systems
School of Engineering Sciences in Chemistry, Biotechnology and Health
KTH- Royal Institute of Technology
“逝者如斯夫,不舍昼夜”
《论语》
Preface

This thesis is submitted to the KTH Royal Institute of Technology in partial fulfillment of the requirements for the Licentiate Degree in Technology. The work has been carried out at the School of Engineering Sciences in Chemistry, Biotechnology and Health (CBH) in Huddinge, Sweden, with Docent Tobias Nyberg (KTH) as main supervisor and Assistant Prof. Xiaogai Li (KTH) as co-supervisor. The research project was supported by funding from the KTH-CSC programme.

The thesis is publicly defended at 9:15, Friday, October 12, 2018, in the lecture hall T67, Hälsovägen 11C, Huddinge, Sweden.
Abstract

It would be of great value to have a precise and non-damaging neuromodulation technique in the field of basic neuroscience research and for clinical treatment of neurological diseases. Infrared neural modulation (INM) is a new modulation modality developed in the last decade, which uses pulsed or continues infrared (IR) light with a wavelength of 1200 to 2200 nm to directly alter neural signals. INM includes both infrared neural stimulation (INS) and infrared neural inhibition (INI). INM is widely investigated for use on peripheral nerves, cochlear nerve fibers, cardiac cells, and the central nervous system. This technique holds the advantages of contact-free and high spatiotemporal precision compared to the traditional electrical stimulation. It does not depend on genetic modification and exogenous absorbers as other optical techniques, such as the optogenetic technique and the enhanced near-infrared neural stimulation (e-NIR). These advantages make INM a viable technique for research and clinical applications. The primary mechanism of the INM is believed to be a photothermal effect, where the IR laser energy absorbed by water leads to a rapid local temperature change. However, so far the details of the mechanism of action potential (AP) generation and inhibition remain elusive. Another issue is that the cells may be endangered by the heat exposure, consequently triggering a physiological malfunction or even permanent damage. These concerns have hindered the transfer of the INM technique to the clinical therapy.

Therefore, the general aim of this study was to improve the understanding of the details of how INM affects the cells. Laser parameters for safe and efficient stimulation were investigated on the basis of being useful for clinical applications. A tailored heating model and in vitro INM experiments on cortex neurons were used to reach this goal.

The first paper was a feasibility study. A 1550 nm laser with a beam spot diameter of around 6 mm was used to irradiate the rat cortex neurons, which were seeded on multi-electrode arrays (MEA) and formed well-connected networks. A heating model based on an estimated laser beam (standard Gaussian distribution) was used to simulate temperature changes. The damage signal ratio (DSR), based on the temperature, was calculated to predict the heat damage. The average spike rate of all the working electrodes from two MEAs was used to evaluate the degree of the inhibition of the neural networks. Results
showed that it is possible to use the 1550 nm laser to safely inhibit the neural network activity and that the degree of the INI is dependent on the power of the laser.

The second paper was an application and mechanism study. The aim of this study was to investigate the safety, efficiency, and cellular mechanism of INI. The same laser as in paper I was used in this study. A 20 X objective was used to decrease the beam spot diameter around 240 µm. The measured laser profile (high order Gaussian beam) was used in the heating model to predict the temperature. The model was verified by local temperature measurements via micropipette. The action potential rates, measured by the MEA electrodes, were quantified for different temperatures. Bicuculline was added to the cortex neuron cultures to induce hyperexcitation of the neural network. The results showed that the INI is temperature dependent and that the temperature needs to be less than 46 °C at 30 s laser irradiation for safe inhibition. The IR laser could also be used to inhibit the hyperexcited activity. The degree of inhibition, for the assessed subpopulation of neurons, was better correlated with the action potential amplitude than the width of it and INI can be accomplished without inhibitory synapses.

**Keywords:** neural modulation, infrared laser, in-vitro experiment, multi-electrode arrays (MEA), heating model, temperature, neural networks, infrared neural inhibition (INI), hyperexcitation
Sammanfattning


Det övergripande målet med denna studie var att öka förståelsen av detaljerna för hur INM påverkar cellerna. Laserparametrar för säker och effektiv stimulering undersöktes med utgångspunkten att de skulle kunna användas i kliniska tillämpningar. En skräddarsydd uppvärmningsmodell och in vitro INM experiment på cortexneuroner användes för att uppnå det här målet.

Den första artikeln är en genomförbarhetsstudie. En 1550 nm laser med en stor strålningspunkt på ungefär 6 mm i diameter användes för att belysa kortikala neuroner, vilka var utsådda på multielektrodmatriser och bildade väl förbundna nätverk. En uppvärmningsmodell baserad på en uppskattad laserstråle (standard Gaussisk distribuering) användes för att beräkna temperaturen. Skadesignalen, baserad på temperaturen, beräknades för att förutsäga värmeskadan. Medlet av aktionspotentialsfrekvensen för alla fungerande elektroder från två MEAer användes för att utvärdera graden av inhibering för
det neurala nätverket. Resultaten visade att det är möjligt att använda en 1550 nm laser för att säkert inhibera den neurala nätverksaktiviteten och att graden av INI är beroende på laserstyrkan.


**Nyckelord:** neural modulering, infraröd laser, in vitro experiment, multielektrodomatriser, uppvärmningsmodell, neurala nätverk, infraröd neural inhibering (INI), hyperexcitering
Acknowledgments

Staying in Sweden for two years to explore the knowledge is one of the most wonderful things in my life. Along with this beautiful journey, so many people have accompanied, supported and helped me. I would like to express my gratitude for some of them.

First and foremost, I would like to express my deepest gratitude to my supervisor, Tobias Nyberg, who allowed me to study freely in the Neuronik lab and guided me to become an independent researcher. Without your expertise, patient, and support, I would never been able to start any experiments and finish this thesis. Thank you for always helping me to find the solution for the problems in the experiments and even in my life, and thank you always taking more time to answer my naive questions and carefully revise my paper and thesis.

I would also like to thank my co-supervisor Dr. Xiaogai Li. You, who are more like a role as a good friend in my life than a supervisor. I got inspired every time when I talked to you. You always show me positive aspects. Thanks to Prof. Svein Kleiven for your support to purchase the experimental devices. Your kindness had a good impact on me. Extend thanks to Peter Arfert, your perfect technical assistance for the experiment setup made my experiment possible.

I would like to express gratitude to all colleagues in the division of the Neuronik KTH. Thanks to Zhou, Madelen, Anna, Pooya, Reza, Shiyang and Teng for the time we spend together. Especially thank to Zhou, you took care of me very well for this two years. You are such a good friend and always listen to all my sorrow and happiness. Thank you always sharing the delicious food and hiking roads with me. I would like to thank Madelen always prepare to help me. Thank you invited me to your parents’ home to have an amazing Christmas day. The cookies, dinner, and gift on that day made me feel so warm. Thanks to Anna, you are a warm-hearted person. Your brave and persistence will encourage me forever. Thanks to Pooya, Raze, and Teng for having so many good time to play badminton together.

I would like to thank my desk neighbor, Abdolamir Karbalaie, you are like my brother from the first day I came to the office. You gave me a lot of suggestion almost for everything. Thanks for your patience to listen to what I wanted to reach and helped me draw so many beautiful pictures for my work. I am so glad to meet your wife and son and I wish you good luck with your defense and future. I would like to thank one of my best friends, Daniel Jörgens, you are an
international bridge of the friendship. Thanks to you organize so many interesting activities, barbecue, mid-summer day, badminton, camping, road trip, and Friday tie day. All these great memories are unforgettable for me.

I would like to thank some of my Chinese friends. Thanks to Hongjian, you are so intelligible. You could always give me some good ideas to solve the problems. Thanks to Ke Lu, you are so patient and smart. Thanks for you taking so many time to listen to my experimental problems, data processing problems and then providing a lot of solutions. And also thank you always encourage me during my studying.

I would also like to express my gratitude to other friends in CBH, Fangyuan, Xin Chen, Liyun, Vinutha..., and in Stockholm, Qian, BochaoWang, Liumin, Yuwei, Yihong, Raugl, Marzieh, Kristoffer, Benny..., and even some people I couldn't remember the name. Thanks to all of you for making this journey more fun.

I would like to greatly thank my family support. Especially thanks to my Father and Mother, you are the great parent! You nerve told me the truth of the reality, the pressure of the life. You always encouraged me to pursue my dream and carefully protected my ambitions. Thanks to other family members, without all of your love and support, I cannot achieve anything.

I would like to thank my supervisor in Chongqing University, Prof. Wensheng Hou. Your support and recommendation provided me a lot of opportunities to pursue my study. Finally, I appreciated the financial support from the KTH-CSC programme.

Qingling Xia

Stockholm, 2018
# Table of Contents

Preface .................................................................................................................. I
Abstract.................................................................................................................. III
Sammanfattning ...................................................................................................... V
Acknowledgments .................................................................................................. VII
Table of Contents ................................................................................................ IX
1 Introduction ........................................................................................................ 1
  1.1 Thesis Outline ................................................................................................ 2
2 Aims ..................................................................................................................... 3
  2.1 Specific Aims .................................................................................................. 3
3 List of Included Papers ....................................................................................... 5
  3.1 Division of Work between Authors ............................................................. 5
4 Background ......................................................................................................... 7
  4.1 Overview of the Nervous System ....................................................................... 7
    4.1.1 The Structure of Neurons and Glial Cells .................................................. 8
    4.1.2 The Production and Propagation of an Action Potential .......................... 9
  4.2 A Brief Introduction to the Laser .................................................................. 12
  4.3 Neural Modulation ....................................................................................... 14
  4.4 Infrared Neural Modulation (INM) ............................................................ 15
    4.4.1 Current Applications of INM ................................................................. 15
    4.4.2 The Biophysical Mechanism of the INM ............................................... 19
5 Methodology ....................................................................................................... 21
  5.1 In Vitro Experiment ..................................................................................... 22
    5.1.1 Cell Culturing ....................................................................................... 22
1 Introduction

Neuromodulation is a technique to modulate and alter neural activity in order to address the disorders associated with the nervous system for therapeutic purposes. This modulation process involves stimulating or inhibiting the nerve signals by using a medical device referred to as neurostimulation device or neurostimulator. The neurostimulation medical device market is one of the fastest growing areas in the medical device industry since the late 1990s [1]. The global neurostimulation devices market size is expected to reach approximately USD 9.8 billion by 2024 (Grand View Research, Inc. report 2018). There are a lot of factors driving the market growth. Some of the factors are a global rise in neurological disorders due to the growing prevalence of lifestyle diseases (such as low back pain and depression) and increasing amount of geriatric population (such as epilepsy, stroke and Parkinson’s disease).

Currently, a number of the electrical neurostimulation medical devices have been developed, such as spinal cord stimulators, deep brain stimulators, and vagus nerve stimulators. The electrical stimulation technique has been the gold standard method for the vast majority of the aforementioned neurostimulation medical device successes. Although a success of these medical devices has been reached, several limitations of the electrical techniques still hamper the patient quality-of-life.

One limitation is the tissue damage due to the contact between the electrodes and the target neurons. Two main types of tissue damage are the mechanical abrasion damage between the nerve and electrode, and the interstitial edema and early axonal degeneration from electrochemical reactions [2]. By choosing a suitable electrode material and stimulus waveform the tissue damage can be limited and chronic safe electrical stimulation is possible. Advances in material composition, biocompatibility, and deposition techniques have been employed to design microelectrodes to mitigate the neuronal death and increase the functional longevity [3]. Another issue is the current spread from the electrode which leads to poor spatial selectivity [4]. Novel high-density silicon microelectrodes have improved local spatial selectivity by reducing the size and increasing the number of electrodes [5]. In addition, researchers are motivated to explore the new modalities [6] for neural stimulation, including magnetic, mechanical, chemical, thermal and optical stimulation.

Neural stimulation using light is not a novel idea. Research on applying visible light on frog muscle tissue and nerve fibers were reported as early as 1891 [7]. During the last decade, infrared neuron modulation (INM) with the wavelength
1200–2200 nm of the laser has been suggested as a method for neural stimulation with several appealing features, such as contactless and no electrochemical junction between the stimulating source and the targets, and finer spatial resolution compared with the electrical stimulation [8]. It does not depend on genetic modification or exogenous absorbers as in other optical techniques, such as the optogenetic [9] and enhanced near-infrared neural stimulation (e-NIR) [10]. Furthermore, a variable depth of tissue penetration can be achieved since several wavelengths can be used in the infrared region [11-13]. These advantages are promising both for research purposes and neuromodulation medical devices.

This thesis, entitled “Infrared Neural Modulation: Photothermal Effects on Cortex Neurons Using Infrared Laser Heating”, aims at an understanding of photothermal effects of infrared neural modulation (INM) on cultures of cortex neurons from the central nervous system and providing guidance for the clinical application.

1.1 Thesis Outline

The thesis is divided into 10 chapters. After this introduction, the aims of this thesis are presented in chapter 2. Chapter 3 includes the references to the two papers on which this thesis is based. Chapter 4 provides relevant background information on the topic of this thesis. Specifically, this chapter introduces the basic knowledge of the neural anatomy and laser techniques, and some main works of the INM. The methodologies used in the thesis are described in chapter 5. The main results are presented in chapter 6 and discussed in chapter 7. The conclusion and future work are presented in chapter 8 and 9. The last chapter contains the references. The full version of the papers is provided in the appendix.
2 Aims

The general aim of this study is to understand and quantify the possible mechanisms and thermal effects of infrared neural modulation (INM) on cultures of cells from the central nervous system, needed to explore the use of INM in clinical applications. This project involved experimental work on INM, biological neural networks and multi-electrode arrays (MEA), and theoretical modeling of the spatiotemporal heating during INM. The results of infrared neural inhibition (INI) were presented in this thesis.

2.1 Specific Aims

- To investigate the feasibility of INM as a modulation technique for inhibiting cortical neural network activity in vitro (Paper I).

- To build a heating model with high accuracy which can be used to predict the temperature characters over the time and space area during IR irradiation (Paper II).

- To qualify the degree of the inhibition by using the temperature dynamics and identify the safe laser parameters for inhibition based on the damage signal ration (DSR) (Paper II).

- To improve understanding of the efficiency and mechanisms of the INI by deeply seeing the thermal effects behind the INI (Paper II).

- To explore the application of the INI by means of pharmacological manipulations (Paper II).
3 List of Included Papers

The thesis is based on the two papers listed below. Full versions of the papers are attached as appendices at the end of the thesis.


3.1 Division of Work between Authors

Paper I  The study design was prepared by QX and TN. All of the experimental work was performed by QX. QX and TN wrote the paper.

Paper II  The study design was prepared by QX and TN. All of the experimental work was performed by QX. QX and TN wrote the manuscript.
4 Background

4.1 Overview of the Nervous System

The nervous system is the most complex and highly organized body system. This system monitors and coordinates internal organ function and directs the body’s reactions to the external environment by receiving, integrating and sending signals to the muscles. The nervous system can be broadly divided into two major parts (Figure 1), the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of the brain and spinal cord, which function to receive, process, and send information to the PNS. The PNS is made up of peripheral sensory and motor nerves. The main function of the PNS is to serve as a pathway of communication between the CNS and the rest of the body.

Figure 1 Diagram of the human nervous system (adapted from Wikipedia). The central nervous system (CNS) consists of the brain and spinal cord and the peripheral nervous system comprises all of the nerves that are not part of the CNS.
4.1.1 The Structure of Neurons and Glial Cells

The nervous system is made up of neurons and glia. The neuron is the basic functional unit of the nervous system, which can receive and transmit chemical or electrical signals to pass information through the body. The extraordinary numbers of the neurons make the nervous system unusual. A human brain alone contains around 86 billion neurons [14]. It is unlikely to identify all types of neurons in the nervous system due to both the technical and conceptual obstacles [15]. Based on the total number of neurites, neurons are classified into three different categories, multipolar, bipolar, and unipolar [14].

Although there are a great variety of types of the neurons, all neurons embody a cell body (soma), dendrites, and an axon (Figure 2). The soma is the spherical central part of the neuron and contains most of a neuron’s organelles, including the nucleus, endoplasmic reticulum, Golgi apparatus, and the mitochondria. The soma is, therefore, the site where major metabolic activities occur. The dimension of neuronal somas varies from 0.004 mm to 0.1 mm in human [16]. Dendrites and axons are branches from the main cell body. Dendrites spread out from the cell body and function as an "antennae" of the neuron to receive signals from other neurons and pass them into the cell body. Incoming signals can be either excitatory or inhibitory (Figure 6). A nerve cell can have many dendrites that are covered by thousands of synapses. The sum of the excitatory and inhibitory signals it receives decides whether or not a neuron is excited into firing an impulse. The site of summation for incoming information is called axon hillock. An axon, a unique tube-like structure only found in the neuron, is responsible for carrying the integrated information away from the soma to specialized endings called axon terminals. The length of axons can vary from 0.1 mm to 3 m [17], dendrites are much shorter than axons.

![Figure 2: The structure of a neuron with a myelinated axon (adapted from [14]). The major components of a neuron are dendrites, soma, and the axon.](image-url)
Glia cells are also vital to nervous system function by playing a supporting role for the neurons. The human brain actually has about 10 times more glia than neurons [14]. There are four main types of glial cells in the human nervous system with the different functions. Three of these, astrocytes, oligodendrocytes, and microglia are in the CNS (Figure 3), while the fourth, the Schwann cells, only exist in the PNS.

Astrocytes are the most numerous type of glial cell in the CNS. The main functions of the astrocyte are providing nutrients and other substances to neurons, guiding the growth of the neurons during their development, maintaining their extracellular environment by regulating the concentrations of ions and chemicals in the extracellular fluid and providing structural support for synapses [14]. Microglias scavenge the dead cells and remove other debris to protect the brain from invading microorganisms. Oligodendrocytes and Schwann cell can generate myelin acting as an insulator around the neural axons, which help them convey the nerve impulse rapidly.

Figure 3  Glial cells support neurons and maintain their environment in the central nervous system (adapted from [14]). Three main glial cells in the CNS are astrocytes, microglia cells, and oligodendrocytes.

4.1.2 The Production and Propagation of an Action Potential

Cellular Membrane and the Generation of the Action Potential

In the nervous system, neurons transmit information by encoding electrical signals (action potential). Electrical potentials are possible due to the excitable cellular membrane of the neurons. Cell membranes contain special protein molecules that span the membrane called ion channels, which are selectively permeable to specific kinds of ions to enter or exit the neuron in the direction of their concentration gradients. Some ion channels are sensitive to the specific stimuli, such as mechanical or thermal. In addition to these ion channels, another set of membrane-spanning proteins, known as active transporters,
establish and maintain ion concentration gradients by moving ions into or out of cells against their concentration gradients. Thus, ion channels and ion pumps work against each other to trigger action potentials and maintain the membrane potential [18] (Figure 4).

In a neuron at rest, the potential inside of the cell is more negative than the outside due to the difference of ions concentration between the intracellular fluid and extracellular fluid. This voltage is called the resting membrane potential or resting potential. A typical resting potential is ranging from -40 to -90 mV and depends on the cell type. Potassium ions (K\(^+\)) and sodium ions (Na\(^+\)) are the two most important ions for the neural signaling. During the resting state, the concentration of K\(^+\) is higher inside and the concentration of Na\(^+\) is higher outside. Both the gated sodium and potassium channels are closed. When the membrane is activated by receiving the neurotransmitters released from the presynaptic neurons (for more details see the section of the synapse), positive ions enter into the neuron and induce more positive potential inside of the neuron than the resting state (phase 1 in Figure 5). If this depolarization continues and increases the potential to its threshold potential (phase 2 in Figure 5), sodium ions enter the cell due to Na\(^+\) channels opening and completely depolarizes the membrane potential referred to as the peak of the action potential (AP, phase 3 in Figure 5). An action potential is considered an all-or-nothing process, also called a spike. Once the AP reaches its peak, Na\(^+\) channels close and K\(^+\) channels open to allow potassium ions to leave the cells. This period is called the refractory period since the sodium channels are inactivated and cannot be opened and no action potential can be generated. The membrane potential becomes even more negative than the rest state, called hyperpolarization (phase 4 in Figure 5). After this point, sodium channels gradually recover to the rest state and prepare to open again (phase 5 in Figure 5).
Figure 5 Five steps for an action potential formation. (1) A target neuron begins to depolarize toward the threshold potential. (2) Depolarization continues and the threshold of the excitation is reached, all the Na\(^+\) channels open. (3) The membrane is completely depolarized and an action potential is produced, the peak is ended by the closing of Na\(^+\) channels and the opening of K\(^+\) channels. (4) K\(^+\) ions continue to leave the cell and hyperpolarize the cell. (5) The K\(^+\) channels close and the cell returns to the resting membrane potential. (Notice: the action potential here is from intracellular recording, which is always positive; the action potentials in my experiments are from extracellular recording, they are always negative (see Figure 19, Figure 21).)

**Synapse and Signal Summation**

One neuron communicates with another neuron by a junction called synapse. Synapses can be divided into electrical synapse and chemical synapse by their mechanism of the transmission. Electrical synapses work by permitting the flow of ionic current and other substances to pass through a gap junction from one neuron to another. The current transmission through the electrical synapse can be bi-directional and extraordinarily fast. These features of electrical synapses generate the rhythmic electrical activity among populations of neurons. Although electrical synapses are discovered in all nervous system, they are in the distinctive minority \[18\]. Compared to the electrical synapses, chemical synapses are more common and function by the release of chemicals known as neurotransmitters. The total number of neurotransmitters is unclear but is more than 100 different types. The target neuron can be either excited or inhibited when the different neurotransmitters diffuse across the synaptic cleft and bind to receptor proteins on the postsynaptic neuron. For example, N-methyl-D-aspartate (NMDA) receptors on the postsynaptic membrane are activated by binding with the transmitter glutamate, which excites the postsynaptic membrane potential called excitatory postsynaptic potential (EPSP). If we consider γ-aminobutyric acid (GABA) as the transmitter, it trends to decrease
postsynaptic membrane potential called inhibitory postsynaptic membrane potential (IPSP). Whether production of the action potential of a neuron or not depends on the summation of the EPSP and IPSP (Figure 6).

![Diagram](image)

**Figure 6** The example of the membrane potential summation (adapted from [18]). Left: postsynaptic membrane potentials depends on the signals of two excitatory synapses (green, E1 and E2) and an inhibitory synapse (red, I). Right: the postsynaptic membrane potentials could be E1/E2, E1+E2, I, E1+I, or E1+I+E2.

### 4.2 A Brief Introduction to the Laser

One of the most important scientific discoveries in the twentieth century has been the laser. It has many unique properties, such as monochromaticity, directionality, coherence, and high brightness. Lasers are applied in a wide variety of areas. The first actual laser was invented by Theodor Maiman in 1960. But the theoretical foundation has been described as early as 1917 by Albert Einstein.

The term of LASER is abbreviated from Light Amplification by Stimulated Emission of Radiation. The generation of the laser is related to the stimulated emission. Based on the theory of the Bohr model, the material can be considered as a quantum system. In this quantum system, electrons orbit the nucleus of an atom at the discrete energy level. For a simple explanation, the effects taking place in the quantum system can be described by two levels only (Figure 7). In general, when an electron is in an excited energy state, it must eventually decay from the higher level (E₂) to a lower level (E₁), releasing a photon of radiation with the energy \( \Delta E = E₂ - E₁ \). This event is called “spontaneous emission,” and the photon is emitted in a random direction, phase, and polarization. On the other hand, according to the quantum theory of radiation by Einstein in 1917, the electron decaying from the energy state \( E₂ \) to the \( E₁ \) occurs when the energy of an incoming photon is approximately \( E₂ - E₁ \). The wavelength, the direction, and the
phase of the new emitted photon are the same as the passing photon. This process is called “stimulated emission”.

\[ \Delta E = \frac{hc}{\lambda} \quad (1) \]

Where \( h \) is Planck’s constant \( h = \sim 6.62 \times 10^{-34} \) Js = \( \sim 4.14 \times 10^{-15} \) eVs and \( c \) is the speed of light in a vacuum \( c = \sim 3 \times 10^8 \) m/s, \( \lambda \) is the wavelength of the laser.

### Table 1

<table>
<thead>
<tr>
<th>Spectral band</th>
<th>Wavelength range</th>
<th>Photon energy range (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>~100-400 nm</td>
<td>~12.4-3.1</td>
</tr>
<tr>
<td>Visible</td>
<td>~400–800 nm</td>
<td>~3.1-1.55</td>
</tr>
<tr>
<td>Near-IR</td>
<td>~800–1200 nm</td>
<td>~1.55-1.03</td>
</tr>
<tr>
<td>Mid-IR</td>
<td>~1.2–7 µm</td>
<td>~1.03-0.18</td>
</tr>
<tr>
<td>Far-IR</td>
<td>~7–1000 µm</td>
<td>~0.18-0.0012</td>
</tr>
</tbody>
</table>

According to the periods of time to generate radiation, lasers commonly can be classified into continuous (cw) and pulsed operation mode. When considering the laser for medical applications, the interaction of light with biological targets is dependent upon the properties of the laser and the optical properties of the targets. The major optical parameters of the laser include wavelength or equivalently the photon energy, laser power, beam spot size or irradiation area,
irradiation time. For the pulse operation model, more optical parameters, such as pulse duration and repetition frequency, need to be considered.

The key parameter for the laser-tissue interaction is the wavelength, $\lambda$. It decides light penetration and distribution in the target and is dependent upon optical properties of the targets, such as refractive index [$n$], absorption coefficient [$\mu_a$], scattering coefficient [$\mu_s$], and anisotropy factor [$g$]. In this thesis, the absorption coefficient and the refractive index of the laser light in the glass and water media for the specific experiment platform was considered. Penetration depth is generally thought of the inverse of the absorption coefficient. Figure 8 is the water absorption coefficients and the penetration depth at the different wavelengths [20]. The wavelength of the laser used in this thesis is 1550 nm. The water absorption coefficient is 10.64 cm$^{-1}$ and the penetration depth in the water is 0.094 cm (940 µm). The 1550 nm IR laser source is cost-effective and has been widely used in the field of the telecommunication, the corresponding photon energy is around 0.8 eV.

![Figure 8](image.png)

Figure 8 The plot shows the water absorption and penetration depth for the irradiation at the different wavelengths (based on data from Hale and Querry (1973) [20], red arrow: 1550nm, red dash box: the common wavelengths for the INM)

### 4.3 Neural Modulation

Neural modulation is the process of activating or inhibiting the excitable neurons or nerves by using an artificial stimulus to evoke or block action potential generation or propagation. Electrical stimulation (ES) technique has been used as a classical neural stimulation technique both in clinically (in diagnostics and therapeutics) [21], and for basic scientific research since the famous experiment in neuromuscular stimulation was performed by Luigi Galvani in the 1780’s [22]. As the method of intraoperative diagnostics, ES has been implemented to
identify the connectivity and functionality of specific nerve roots in peripheral nerve surgery [23]. As the mapping method, ES has been used to map the cortical surface of the motor and sensory areas during brain tumor resection and map deep brain structures during the deep brain electrodes implantation [24, 25]. As the method of treatment, ES treatment could relieve pain [26] and could be beneficial to the Parkinson’s disease or other movement disorders or some types of the neurological disorders [27].

4.4 Infrared Neural Modulation (INM)

Although the widespread use of the ES, it has several fundamental limitations. One is the necessity of the physical contact between the electrodes and target areas, which may induce tissue damage. Second, the spreading of the current results in the undesired response and a relatively poor spatial selectivity. Third, a “stimulus artifact” produced by the electrical stimulation interferes with recording response around the stimulation site.

To overcome the limitations of ES, the infrared neural modulation (INM) has been proposed as an alternative to ES by several groups. INM is a label-free method by employing the infrared (IR) laser to elicit and inhibit the neural activity with the advantages of contact-free, damage free and high spatial resolution. INM includes both infrared neural stimulation (INS) and infrared neural inhibition (INI). Both INS and INI work through the mechanism of the IR-induced heat. INS is considered to be a spatiotemporal gradient (dT/dt and dT/dx) [28, 29], while INI is believed to be an increase in baseline temperature (ΔT). The IR wavelength 1200-2200 nm has been widely employed to control neurons activity in the peripheral nervous system [30-33] and the central nervous system [34-36].

Historically, numerous studies using light to interact with the neural system can be found in the literature [37-39]. However, the first report that action potentials in neurons could be induced by the optical irradiance was published in 1971 [40]. Systematic studies of using infrared lasers to generate neural activation were reported by the Wells et al. [29, 41] after their first report was published in 2005 [30].

4.4.1 Current Applications of INM

IR in Peripheral Nerves

Sciatic Nerve

The first successfully INS application of the nerve activation was reported by Wells et al. [30]. Six wavelengths, 2.1, 3.0, 4.0, 4.5, 5.0 and 6.1 µm, at the repetition rate 1-30 Hz were used to stimulate the rat sciatic nerve. Both compound nerve action potentials (CNAP) and the compound muscle action
potentials (CMAP) were recorded accompanied by muscle contraction. In their study, the minimum radiant exposure required for a visible muscle contraction was defined as stimulation threshold, and the minimum radiant exposure required for visible cavitation or ejection of material from the nerve was defined as damaging ablation threshold. The ratio of damaging ablation threshold and stimulation threshold was referred as safety ratio. Stimulation and ablation thresholds exhibit a function of wavelength-dependent water absorption coefficient. They found that the most appropriate and safest wavelengths were 2.1 μm and 4.0 μm with the safety ratio of 5.5 and 5.0 among the above six wavelengths. Furthermore, the CMAP only occurred when the laser directly illuminated the nerve fascicle innervating that muscle, which implied a higher spatial specificity than electrical stimulation. After the first success of INS study, Wells et al. have continued a series of systematical studies on the rat sciatic nerve [29, 41, 42]. Their later safety study shows the nerve damage occurring at radiant exposures above 2 times the stimulation threshold at the lower repetition rate [41]. Their biophysical mechanism study shows the temperature gradient at the axon level (3.8–6.4 °C) main responsible for the neural activation during the pulsed laser light [29].

McCaughey et al. [43] also have observed the leg twitch and muscle activation by using four different lasers (2100, 1495, 1450 and 1540 nm) on the rat sciatic nerve. Their experiments suggested that the most effective stimulus is 1495 nm laser with a single mode fiber.

**Cavernous Nerves**

IR Laser also has shown the potential application of cavernous nerves. Fried et al. [32] have increased the intracavernosal pressure of the rat cavernous nerves by using 1870 nm laser. In addition, Tozburun et al. [11, 44] reported 1455, 1490, and 1550 nm laser could evoke the cavernous nerves response with the fascia thickness 100-110, 360-380 and 420-450 μm. They demonstrated that the 1490 nm laser is possible to provide rapid and sufficiently deep subsurface stimulation for the rat prostate cavernous nerves.

The effects of the combination of ES and IR on peripheral nerves were studied by several groups [45-50]. Duke et al [45] demonstrated the ES could be used to lower the threshold of the INS, increasing the safety ratio from 2:1 to 6:1. The results from Peterson et al. [48] indicated the INS alone could locally and directly activate the small nerve fibers out of a population of the axons. They also found that the combination of the ES and INS might decrease the electrical excitability. This recruitment inhibition behavior is similar to the action potential inhibition reported by Mou et al. [49] and Duke et al. [51].
IR in the Ear

The unique advantages of INM make it an alternative technique in auditory neural activation, which could be beneficial to the cochlear implants (CIs). Infrared neural modulation (INM) of the cochlea has been investigated in varying animal models, including gerbil [52], mouse [53], guinea pig, and cat [54]. Studies were conducted in normal-hearing, acutely deafened [33, 55, 56], and chronically deaf animals [57].

Izzo et al [33] first showed that an IR (λ=2120 nm) could be used to stimulate the auditory nerve. Optical evoked compound action potentials (oCAPs) and spiral ganglion cells were stimulated directly. No neural damage occurred after 6 hours of continual IR stimulation. Responses were found in both normal-hearing and in deaf animals missing all hair cells. Recently, Tan et al. [58] also demonstrated that hair cells are not required for the auditory brainstem responses during the IR irradiation which instead directly interacts with spiral ganglion neurons (SGNs). Richter et al. [57] reported, compared to the acutely deafened deaf gerbils, the optical thresholds of the chronically deafened deaf gerbils for producing CAPs were not significantly elevated for pulse durations shorter than 100 μs. However, significant threshold elevations occurred for pulses of 200 μs or longer. These threshold elevations were believed to be correlated with the less number of surviving spiral ganglion cells.

Moreover, the variable optical parameters including wavelength (λ=1844-1940 nm), pulse duration (τp=5 μs-1 ms) and repetition rate (f=2-1000 Hz) were investigated [59, 60]. The results show that the pulse length shorter to 5 μs can evoke the CAPs and longer pulse needs to deposit more energy to achieve the stimulation. Thus, they suggested the pulses in the range of 1-100 μs seemed ideal. In addition, a single auditory nerve fiber response to optical stimulation was reported by Littlefield et al. [52]. Their results showed that the neurons had higher firing efficiencies at stimulation rates less than 100 Hz and decreased drastically at higher rates.

The spatial selectivity of the INM in the auditory nerve was verified in different ways. Immunohistochemical staining for the protein c-FOS [13] showed that the only small area of neurons was active under the optical radiation. This area was within the optical path. The pattern of the c-FOS staining was similar to acoustic stimuli and contrasted to the electrical stimulation. A masking method was used to demonstrate the spatial selectivity [61]. The width of the tone-on-light tuning curves was similar to the width of tone-on-tone tuning curves, which indicated the fine spatial area of INM. The inferior colliculus (ICC) response during the IR was compared to the response from the acoustic tone in [62]. The results also showed the response area in the ICC with IR was similar to that obtained from acoustic tones.
INM has been explored on the vestibular nerve system by several researchers. Harris et al. [63] evoked the compound nerve potentials of the vestibular and facial nerve by using IR with the wavelength of 1842 nm. Superior and lateral vestibular semicircular canals could be selectively stimulated. In [64], physiological eye movements could be frequency modulated by the IR pulsed at the posterior semicircular canal.

**IR in the Central Nerve System**

Cayce et al. [34] first showed the feasibility of INM in the CNS by activating cortical neurons in thalamocortical brain slices. The evoked neuron response was frequency locked with the pulses and the INS was wavelength and frequency dependent. The primary results that the IR could evoke the cortical neural potentials in vitro motivated the first in vivo application of INM in the rodent somatosensory cortex. In [12], the neuronal activity evoked by the IR was detected by optical intrinsic signal imaging (OISI) technique, in which neuronal activity is measured by imaging light reflectance changes in the blood. Their results showed that the OISI signals could be modulated by varying repetition rate and radiant exposure of the IR. They also found the inhibition of the spontaneous neural activity by using IR for the first time. The reason of the INI was not fully understood, although they proposed it was related to the large beam size (diameter 400 µm), which might excite inhibitory neurons [35]. Furthermore, evoked calcium signals including fast component and a slow component in vivo under the IR were observed in their other study [36]. To move forward the clinical application of the INM, the cerebral cortex of non-human primate, which is the closest animal model to the human cortex, was studied by the same group [35]. For the first time, INM can directly modulate the functional response on the non-human primates in vivo and they found that the inhibition effects were related to the spot size due to the special anatomical organization of the visual cortex.

The systematical investigation by Caye et al. of the INM in the CNS showed the complexity of IR evoked cortical activity. Their results demonstrated not only excitatory and inhibitory neurons but also non-excitable cells, such as astrocytes, are involved in the INM. While, INM in the CNS shows the same contact-free, artifact-free, and spatial precision characteristics reported by researchers using INM in the PNS [60],[31],[32, 42].

**IR in the Cardiac Pacing**

The first optical pacing of an intact heart in vivo by using infrared light was reported by Jenkins et al.[65]. A pulsed diode laser (λ=1875 nm) with the frequency 2-3 Hz elicited a synchronized heartbeat on the embryonic quail hearts. The study was further developed in the adult rabbit heart [66]. A pulsed
(λ=1851 nm) laser with the pulse duration 2.5-12 ms and the frequency 2.5-3.3 Hz successfully paced the adult rabbit heart rate. In addition, extended pacing (1-5 minutes) was achieved. The stimulation threshold was related to both the pulse duration and pulse frequency. Wang et al. [67] also successfully paced quail hearts and mouse hearts by employing the 1440 nm and 1465 nm laser. The first infrared cardiac inhibition was reported in 2016 [68]. It demonstrated that IR could be used to stop contractions by inhibiting action potentials with high spatial precision, which leads to the abolition of intracellular calcium transients. In addition to the investigation on the hearts, studies on cardiomyocytes, subjected to IR irradiation, were also reported by other groups [69, 70].

### 4.4.2 The Biophysical Mechanism of the INM

Effects of light on biological tissues can be photochemical, photothermal, photomechanical, and photoelectrical, depending on the size and nature of the sample as well as the wavelength of the light. Although the mechanism of INM was not fully understood, most studies reported that both INS and INI worked through the mechanism of the IR-induced heat. INS was proposed as a photothermal effect by several groups, with an estimated temperature rise of 0.1 °C [60], of 4–6 °C [29], of 14 °C [71], of 22 °C [28], or of 13-35 °C [72]. Water absorption of the optical radiation is considered as the source of the IR heating modulation [28, 29, 60].

In 2007, Wells et al [29] first investigated the behind mechanisms of INS by using Ho: YAG laser (λ=2120 nm), free electron laser (λ=2100 nm) and diode laser (λ=1870 nm) to irradiate the rat sciatic nerve. Photochemical effects required the high energy of the photon, while infrared photon energy was too low to directly drive photochemistry in their study. Photomechanical effects are involved with the pulse length shorter than 1 µs. However, the minimum pulse length used in their study was 5 µs. Thus, they excluded photochemical and photomechanical mechanisms and supported the water absorption driven photothermal process that requires a transit thermal gradient on the nerve axon level.

Following the proposal of the heat-sensitive ion channels worked behind INS, the heat-sensitive vanilloid subfamily of TRP channels (TRPV) as the one potential mechanism of INS has been reported in the several papers [53, 72, 73]. TRPV channels are found in the whole nervous system and they are nonselective cation channels permeable to Na+, K+, and Ca2+ [74]. Different TRPV channels are activated by different temperature thresholds. The temperature thresholds for the HEK 293 cells and Xenopus oocytes as follows: TRPV4 >27°C, TRPV3 >32°C, TRPV1 >43 °C and TRPV2 >52°C [73]. Albert et al. [73] demonstrated that IR laser evoked retinal ganglion cells (RGCs) and vestibular
ganglion cells (VGCs) due to the TRPV4 activation. In their study, the local temperature rise (10±2 °C, 7 ms) induced by the laser irradiation was reported as suitable for TRPV4 activation. Suh et al. [53] reported that IR failed to evoke the auditory nerve response of mice lacking the gene for the TRPV1 channel, which also illustrated that TRPV channels play a role in the INS.

Shapiro et al. [28] showed a general mechanism of the heating altered the membrane capacitance by using IR lasers on the Oocyte, bilayer and HEK cells. The measured temperature increased 22 °C and measured capacitance increased up to 6% during the 10 ms IR pulsing. The current arising from temperature-dependent capacitance changes predicted by the classical Gouy–Chapman–Stern (GCS) model [75] was consistent with their experimental measurement. To demonstrate the important role of the water, the standard water was replaced by the heavy water in their experiment. The observed response was reduced by 80% as the absorption coefficient of heavy water is approximately 20% of the standard water at the wavelength of 1889 nm.

Intracellular calcium transient [69, 76, 77] was demonstrated as one of the possible mechanisms of IR. Dittami et al. [69] used IR to irradiate neonatal rat ventricular cardiomyocyte and IR-evoked [Ca^{2+}]_i events were observed. Mitochondria as the major intracellular store of Ca^{2+} was pointed in the IR-evoked responses according to their pharmacological array studies. Recently, pulsed IR-induced Ca^{2+} signals were investigated by using IR on the HT22 mouse hippocampal neurons and U87 human glioblastoma cells [76]. The results showed that the IR-induced Ca^{2+} transients was from intracellular stores and mediated by phospholipase C and IP3 signaling pathway. An in vivo study in the central nervous system [36] also supported that the mechanism of the INM is related to calcium transients. The two component calcium signals revealed that the multiple cellular processes (apical dendrites, interneurons, and astrocyte processes) were involved in the INM. The studies combined the two 2-photon imaging method and pharmacological method confirming activation of astrocyte networks and activation in dendrites, axons, and glial processes during the INM.

Other possible mechanisms of the IR-induced the response include the neurotransmitter release (GABAergic neurotransmission) [78] and the formation of nanopores [79, 80]. Thus, the studies of the multiple cellular mechanisms for INM modulated neural signals from different groups suggest various complex mechanisms for the effects on cells.
5 Methodology

The general aim of this thesis was to figure out the relationship between the laser parameters, temperature, and the neural response. This chapter gives an overview of the methods used in the research study, i.e. modeling of the heating during laser irradiation and in-vitro experiments using cultures of rat cortex neurons to investigate the biological responses caused by the laser heating. For two papers in this thesis, we used the same experiment platform and cells. The main differences are presented as following:

- The laser spot size diameter was decreased from the 6 mm to the 240 um by using a 20 X objective lens in paper II.
- The laser beam profile is a high order Gaussian distribution in paper II and a standard Gaussian distribution in paper I.
- The temperature model was improved in paper II.

Table 2 Overview of the studies in this thesis.

<table>
<thead>
<tr>
<th></th>
<th>Paper I</th>
<th>Paper II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aim</strong></td>
<td>Laser power vs Neural inhibition</td>
<td>Temperature rise vs Neural inhibition</td>
</tr>
<tr>
<td><strong>Wavelength</strong></td>
<td>1550 nm</td>
<td>1550 nm</td>
</tr>
<tr>
<td><strong>Laser beam</strong></td>
<td>Standard Gaussian</td>
<td>Higher order Gaussian</td>
</tr>
<tr>
<td><strong>Operation model</strong></td>
<td>Continues wave</td>
<td>Continues wave</td>
</tr>
<tr>
<td><strong>Spot diameter</strong></td>
<td>6 mm</td>
<td>200 um</td>
</tr>
<tr>
<td><strong>Laser power</strong></td>
<td>90, 180, 270, 340 mW</td>
<td>2, 15, 28, 42, 56 mW</td>
</tr>
<tr>
<td><strong>Irradiation time</strong></td>
<td>60 s</td>
<td>30 s</td>
</tr>
<tr>
<td><strong>Recording tools</strong></td>
<td>Standard MEAs</td>
<td>Standard MEAs</td>
</tr>
<tr>
<td><strong>Cells</strong></td>
<td>Rat cortex neurons</td>
<td>Rat cortex neurons</td>
</tr>
</tbody>
</table>


5.1 In Vitro Experiment

5.1.1 Cell Culturing

Primary cortex neurons used in two papers are from an embryonic day 18 (E18) Sprague Dawley (SD) rat cortex, which was purchased from Brainbits of UK. The dissociation procedure was followed the BrainBits Primary Neuron Culture Protocol.

Specifically, after cleaning and sterilizing the multi-electrodes arrays (MEAs), 100 μl of 1 % polyethyleneimine (PEI, Sigma, USA) solution was applied to the center of the MEAs at room temperature overnight. Before use, the MEAs were rinsed with sterilized ultrapure water and allowed to dry. Dishes were covered 20 μl mouse laminin in medium (20 μg/ml, Invitrogen) for 1 to 2 hours in the incubator (37 °C, 5 % CO2) before adding the cell suspension.

Cortex was dissociated with papain (2 mg/ml) for 20 min at 30 °C, followed by centrifugation at 250 G for 1 min. Cell culture medium (NbActive4, BrainBits) was used. Half medium (NbActive4, BrainBits) was changed every 3 days. Electrophysiological recordings were performed about 3-4 weeks after seeding. Figure 9 shows cortex neurons on the MEA after 0, 6, and 24 days seeded. After 6 days seeded, the neurons have formed a well-connected network (Figure 9 B, E), although there is no spontaneous activity.
Figure 9 Dissociated cortical neurons cultured on the multi-electrodes arrays (MEAs) at the different days after seeding. A: 0 DIV. B: 6 DIV. C: 24 DIV. D, E, F are the magnification views of the center of the A, B, C. DIV is the abbreviation of days in vitro. The electrode diameter is 30 μm; electrode spacing is 200 μm.

5.1.2 Experimental Setup of Primary Cortex Neurons Stimulation

As aforementioned, neurons were cultivated on standard multi-electrode arrays (MEAs, Multichannel Systems, Reutlingen, Germany) having 60 electrodes with an electrode diameter of 30 μm and an inter-electrode distance of 200 μm using silicon nitride to insulate the leads. The neural signals can be directly detected by
the electrodes of the MEAs. More details about the electrophysiological recording will be introduced in the electrophysiological recording section.

Figure 10 illustrates the schematic of IR irradiation in the two papers. A same infrared laser source (Modulight, Tampere, Finland) with the wavelength 1550 nm was used in the two papers. Laser power used in the experiments was measured by a Thorlabs S302C power meter (Thorlabs, Newton, NJ).

In the paper I, laser light illuminated the cells after it passed through the collimator (F220SMA-1550, Thorlabs, Newton, NJ) and a custom mirror. The spot diameter is around 6 mm and the laser intensity profile was assumed as a standard Gaussian beam.

In the paper II, the light after the collimator and mirror was focused with a 20X objective (Leica, Germany) to yield a focused IR beam spot with a diameter of around 240 μm. The laser intensity profile was measured as a four order Gaussian beam distribution around 1 mm of the focus layer.

![Figure 10](image)

Figure 10 The illustration of the experimental setup for IR irradiation in the paper I (A) and in the paper II (B). The same laser source was used in the two papers.

5.1.3 Electrophysiological Recording

The same electrophysiological recording method was used in the two papers. Neural activity was recorded by the MEA at the age of 19-27 DIV (days in vitro). The MEA was placed in an MEA-1060 Headstage (Multichannel Systems, Reutlingen, Germany) and heated to a baseline temperature. The electrode signal was amplified by a 16 channel amplifier (Micro Electrode Amplifier, model 3600, AM Systems) and digitized using DataWave software (SciWorks, Loveland, CO v. 6.0).

Before the stimulation experiments, MEAs were taken out from the incubator to check for activity in the form of spontaneous bursts and single action potentials.
Laser irradiation experiments were only conducted on MEAs having spontaneous activity.

5.1.4 Pharmacological Manipulations (in paper II)

We applied the GABA_A receptors antagonists bicuculline (concentration, 20 μM, Sigma) to the cortex neuron cultures in order to induce the hyperexcitation of the neural network activity. The drug mixture was directly added onto the culture using a micropipette. After that, neurons were exposed repeatedly under the IR laser (spot diameter around 6 mm, laser power density 14.5 mW/mm²).

5.2 Heating Model

To a better understanding of temperature effects during infrared neuron stimulation, a heating model was built by multiphysics software from COMSOL (COMSOL Multiphysics, 2016, version 5.2a, Stockholm, Sweden) in the two papers. Two models share the same basic theories. We discretized the model as cubic elements and considered the interface non-isothermal flow, a multiphysics coupling of fluid flow and heat transfer. The two main components for heat transport in the model are conduction and convection. The differences between the two models are in Table 3. The 3D geometry of the models was used in the two papers in Figure 11 and the dimensions of the models are in Table 4.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validated</td>
<td>None</td>
<td>Validated</td>
</tr>
<tr>
<td>Laser beam function</td>
<td>$\gamma(z) = e^{-2 \left( \frac{\sqrt{x^2+y^2}}{w} \right)^2}$</td>
<td>$\gamma(z) = e^{-2 \left( \frac{\sqrt{x^2+y^2}}{c} \right)^4}$</td>
</tr>
<tr>
<td>Geometry</td>
<td>A small area of the MEA chamber (Figure 11 A)</td>
<td>Whole MEA chamber (Figure 11 B)</td>
</tr>
<tr>
<td>Boundary condition</td>
<td>Topside: 32 °C Bottom side: 32 °C Others: outlet or outflow</td>
<td>Topside: convective cooling Bottom side: 37 °C Others: 22 °C (but convective cooling in the center)</td>
</tr>
</tbody>
</table>

In two papers: $w, c$ was set as 0.817 times of their beam radius.
Table 4 Dimensions of the models in the two papers.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass ring</td>
<td>The inner diameter 19 mm, outer diameter 24 mm, height 6 mm</td>
<td>none</td>
</tr>
<tr>
<td>Water</td>
<td>Diameter 19 mm</td>
<td>Diameter 2 mm</td>
</tr>
<tr>
<td></td>
<td>Height 3.5 mm</td>
<td>Height 0.5 mm</td>
</tr>
<tr>
<td>Finer mesh water</td>
<td>Diameter 2 mm</td>
<td>Diameter 0.5 mm</td>
</tr>
<tr>
<td></td>
<td>Height 1.5 mm</td>
<td>Height 0.5 mm</td>
</tr>
<tr>
<td>Glass substrate</td>
<td>Diameter 24 mm</td>
<td>Diameter 2 mm</td>
</tr>
<tr>
<td></td>
<td>Height 1 mm</td>
<td>Height 1 mm</td>
</tr>
<tr>
<td>Finer mesh glass substrate</td>
<td>Diameter 2 mm, Height 1 mm</td>
<td>none</td>
</tr>
</tbody>
</table>
Figure 11 The 3D geometry models used in the paper I (A) and paper II (B).
Table 5 Parameters Used in the Model and experiment during the IR irradiation.

<table>
<thead>
<tr>
<th></th>
<th>Calibration</th>
<th>Experiment</th>
<th>DSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser power (mW)</td>
<td>I</td>
<td>None</td>
<td>90, 180, 270, 340</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>164</td>
<td>2, 15, 28, 42, 56, 70, 83, 97, 111</td>
</tr>
<tr>
<td>Stimulation time (s)</td>
<td>I</td>
<td>None</td>
<td>60#</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5</td>
<td>30*</td>
</tr>
<tr>
<td>Initial temperature (°C)</td>
<td>I</td>
<td>None</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>22</td>
<td>37</td>
</tr>
</tbody>
</table>

To save the computation time, # simulate 3 seconds in the model to predict the 60 seconds the temperature rise in the experiment; * simulate 2 seconds in the model to predict the 30 seconds the temperature rise in the experiment. DSR: Damage Signal Ratio

5.3 Local Temperature Measurement

Temperature measurements resulting from infrared laser irradiation were made by using open glass pipette (diameter 10 μm, resistance 1–3 MΩ), following the method of Rickard et al. [81] and of Yao et al. [72]. Calibration was obtained by heating the saline solution to about 60 °C and by passive cooling to 25 °C while simultaneously recording the tip current (Ag/AgCl electrode) of the open pipette and the temperature of the solution using a PT-100 thermistor (Figure 12 A). A linear curve was fit to the absolute temperature versus the logarithm of pipette current by using MATLAB (Figure 12 B).
Figure 12  A: The illustration of temperature calibration. B: The linear curve of the solution temperature and pipette current. R-square 0.999, fitting numbers, n=6501.

After calibration, the pipette was used to monitor current change during the laser irradiation in order to verify the temperature behavior predicted by the model. These temperature measurements were performed at room temperature (22 °C) and the experimental setup for measurement is shown in Figure 13. A glass chamber (the same size as the chamber of an MEA) with 1 ml saline solution was placed on the stage of the microscope (Leica DM Inverted Light microscope, Leica Microsystems, Switzerland) and a 20 X objective was used to focus the laser source. The tip of the pipette was positioned near 100 μm above the substrate using the XYZ translation stage (Thorlabs, US). Then by moving the XYZ translation stage with 10 μm along the x, y-direction and following a 5 seconds laser irradiation for each step to find the center of the laser beam. After the beam center was recognized, the pipette was moved radially along the x-axis in 10 μm step through the center.

To easy monitor the current change, a higher laser power of 164 mW was used in the measurement to induce the higher current change. The launched laser power after the objective into the water was estimated about 5% loss due to the glass reflection.
Figure 13 Photograph of temperature measurements. A: Overview of the experimental setup. B: The glass chamber (the green dash box in the A). C: The tip of the glass pipette (the purple dash box of the B).

5.4 Data Analysis

The procedure of the signal processing is shown in Figure 14. To analyze and extract the spikes, digitized signals were filtered with a Butterworth band-pass filter (20-2000 Hz). The detection threshold was set to 1.5 times than the noise level using the software of the DataWave (SciWorks, Loveland, CO v. 6.0). Then the extracted data was imported to the MATLAB to eliminate any false positives. To eliminate false positives due to artifacts, the signal was removed with a time window of 2 ms before and 8 ms after the laser pulse turned on point and with a time window of 3 ms before and 2 ms after the laser turned off point. The other false signals were eliminated by setting the threshold of the spike amplitude (> 10 µV) and the width of the spike (240-720 µs).
For the analysis of neural activity, average spike rates were calculated before, after, and during laser stimulation (bin=1 s). To evaluate the degree of the neural activity inhibition, we only chose the active channels with average spike rates.
more than 5 spikes per seconds before the laser stimulation. The spike rate change (SRC) with or without the IR irradiation was estimated by the following equation 2.

\[
\text{SRC}(\%) = \left( \frac{\text{spike count during stim} - \text{spike count before stim}}{\text{spike count before stim}} \right) \times 100\% \quad (2)
\]

All statistical data were presented in mean and standard deviation (mean ± SD).

### 5.5 Safety Analysis

We evaluated the safety of our laser experiments by calculating the damage signal ratio (DSR, Equation 3, 4) before we start the experiment, which was based on previous hyperthermia research for cerebral cortex cells in our group [82].

\[
\text{DSR} = 1 - \frac{C_1}{C_0} \quad (3)
\]

\[
\int_{0}^{t} A_c \cdot e^{-\left(\frac{E_a}{R \cdot T(t)}\right)} \, dt = -\ln \frac{C_1}{C_0} \quad (4)
\]

C₀, initial concentration of an unspecified internal chemical component; C₁, remaining concentration of chemical component after the neurons exposed by the laser; Aᵣ, frequency factor (9.76×10⁻⁵⁰ s⁻¹); Eₐ, reaction activation energy (333.6 kJ/mol); t, time of IR irradiation (60 or 30 s); T(t), temperature during the IR irradiation (K), R the gas constant (8.314 J/(K·mol)).
6 Results

The main results of the two papers are shown in this thesis. Additional results can be found in the articles in the appendix.

6.1 Paper I - Photothermal Inhibition of Cortex Neurons Activity by Infrared Laser

The temperature model predicted the temperature changes over the time in the center of the laser beam at output powers of 90–340 mW in Figure 15. The cortex neurons were repeatedly stimulated 3 times by turning the laser on 60 seconds and off 180 seconds as Figure 16. When the laser power increased from 0 to 340 mW, for MEA1 and MEA2, the degree of suppression rose monotonically from 0 to 88.18 % and from 0 to 62.96 % respectively (Figure 17). The low DSR shows there is no damage happened in the experiment.

Figure 15 Temperature distribution on the cell layer (0.1 µm above the MEAs substrate) at the different laser power (Laser power: 90 mW, 180 mW, 270 mW, and 340 mW; Laser wavelength: 1550 nm; Initial temperature 32 °C). A: Temperature distribution in the center of the laser beam at the different laser power over the time. B: Temperature distribution at the distance from the center along the x-axis at 2 s.
Figure 16  Laser irradiation scheme and single channel suppression illustration. A: The schematic of 3 times repeated laser irradiation.  B: The raw data of repeating suppression of spontaneous neural firing from a single electrode (laser power 270 mW, MEA 1).

Figure 17  Quantification of spike rate changes (SRC, mean±SD) of two MEA samples at the different laser power.
6.2 Paper II - Inhibition of Cortical Neural Networks Using Infrared Laser

The temperature model was verified by the local temperature measurement, which showed that the difference between simulation and measurement is less than 2 °C (data not shown). The model predicted that the maximum temperature induced by the laser at the cells layer is from 37.4 to 53°C with the laser power from 2 to 111 mW, corresponding to the temperature rise from 0.4 to 16 °C (Figure 18).

![Temperature distribution at the cells layer (0.1 µm above the glass substrate). A: Temperature distribution in the center of the laser beam at the different laser power over the time (initial temperature 37 °C). B: The peak temperature rises with increasing laser power.](image)

The damage signal ratio (DSR), which is relating to both exposure temperature and exposure time, is 2.9 % and 5.5 % by using 30 s IR irradiation with laser power 83 and 97 mW (Figure 19 A). The value of the DSR shows that neurons are safe under the condition that the laser power was 83 mW. However, the results from the experiments implied that the functional activity of the neurons was damaged due to the mean shape of the spike was distinctly changed after the 83 mW laser power irradiation (Figure 19 B).
Figure 19 A: The DSR (magenta diamond) and maximum temperature (blue diamond, 0.1 µm above the glass substrate) at the laser power 2, 15, 28, 42, 56, 70, 83, 97 and 111 mW for 30 s IR irradiation. (Initial temperature is 37 °C, the black dash line represents the damage threshold). B: The average shape of the spike before the laser and after the laser.

The spike rate changes (SRC) plot in Figure 20 displays that all neurons could be inhibited with the increased temperature at the temperature range of 37.4-46 °C, even though some neurons would become more active for a slight temperature increase.

Figure 20 The spike rate changes (SRC) from a single electrode in different MEAs at the different temperatures (2 groups, laser power 2-56 mW, temperature 37.4-46 °C). All values represent mean ± SD, repeat irradiation times n=3 for each point. Red cross: the activity of the cells did not recover.
In our experiment, a single electrode commonly records more than one type of action potentials characterized by different spike amplitudes. Both the small and large amplitude spikes followed the same trend, with increasing pulse power the spike amplitude became smaller, the spike width narrower, and there was a prominent increase of the recorded potential at the end phase of the action potential (Figure 21). Figure 22 shows that the hyperexcited bursts were suppressed during the IR and recovered after the IR. The results suggested the epileptiform activity could be also inhibited by the IR.

Figure 21  The average shape change of the action potentials before (blue, 30 seconds), during (red, 30 seconds) and after (green, 30 seconds) the different laser power irradiation. A: The average shape of small amplitude (less than 20 µV) spikes at the laser power 2, 15, 28, 42, 56 mW (from the left to right); B: the average shape of large amplitude (larger than 20 µV) spikes at the laser power 2, 15, 28, 42, 56 mW (from the left to right). (Same data as the magenta diamond in Figure 20).
Figure 22 Epileptiform neural activities inhibited by the IR. A: Different channels respond to the bicuculline (black arrow) and IR laser exposure (the red bar). B: Bicuculline induced the epileptiform neural activities, magnify from the first blue box of the A. C: Epileptiform neural activities reduced by 70% under the IR, magnify from the second blue box of the A. (14.5 mW/mm²; irradiation time, 20 s; spot diameter, 6 mm; repeat times, 6)
7 Discussion

This thesis is based on the model of the heating and cellular experiments of the neural response during the INM. The model helps to understand the spatial and temporal temperature distribution at the cellular level in a micro-volume when we applied different laser parameters, such as the laser power and irradiation time. After clarifying the heating process, the modulation efficiency and the safety of the neural activity by the INM could be interpreted from the temperature aspects.

7.1 Modeling

Temperature is a crucial parameter for the application of the INM. Not only because heating of the neural targets induced by the infrared laser can excite [28, 29, 57] or block [50, 83] the activity of the neuron, but also an excess deposit of the energy could potentially cause damage [82, 84] to the neural targets. Thus, it is important to precisely monitor and measure the thermal changes during INM to better control the neural response and to avoid the heat-induced injury. However, all the developed measurement techniques have presented some challenges for the microscopic scale system and complexity of biological environments [85]. In this case, modeling is a powerful tool to understand the thermal dynamics during the INM. In the cochlea, Thompson et al. [86, 87] built a detailed temperature model, which was based on the Monte Carlo method to calculate the spatial distribution of light absorption and a finite element solution to solve the heat equation. Their model could be applied to peripheral nerves, the cochlea, and in vitro geometries to predict the temperature behavior.

It is feasible to use a model to predict a relativity accurate temperature behavior during INM in our studies. The experiment platform is relatively simple in our study. The chamber of the MEA includes a regular geometry including a glass substrate, a glass wall, and water. The normalized laser beam distribution used in our study can be easily captured by a camera in the air. The measured laser beam profile shows a higher order Gaussian distribution around 1mm of the focus plane and a standard Gaussian distribution further away along the beam axis in the air, which is consistent with the previous results [88]. Absorption, reflection and refraction of the light by the different layers of the MEA were then used to adapt the beam in the air to the final beam in the culture dish. Light scattering was ignored as besides a thin cell layer light beam is mostly passing through non-turbid media.
The accuracy of the model is essential to trust the simulation results. This model was verified by the open pipette method under the 5 seconds IR pulsing at the laser power 164 mW. Both the temperature simulation and local temperature measurement shows the temperature equilibrated around 60 °C in the 2 seconds during the IR, although the measurement has slightly increased after the 2 seconds. The temperature difference between the simulation and the local measurement is less than 2 °C in the time domain and spatial domain within 400 µm diameter (data not shown). To save calculation time, the equilibrium temperature at 2 seconds of simulation was used to predict the temperature equilibrium for the 30 second pulse.

This heating model can be also easily adapted, especially for the IR irradiation pattern. We can easily change the pulse lengths, frequency, energy and irradiation time to calculate the temperature. We also expect this heating model theory could be adapted to other types of experiment operation model with similar geometries, such as optogenetic stimulation in vitro based on the microscopy system.

As the above statement, this model was tailored to predict the temperature profile in our experiment. The difference between model prediction and temperature measurement is less than 2 °C and it is easily adapted. However, there are also some limitations to this model. The key limitation of the model is highly sensitive: the simulation results are easily affected by the geometry boundaries, mesh size and time steps. To decrease the boundary effects, a geometry having the same size as the chamber was used. The mesh sizes of coarse, fine, finer, extra fine and user-defined sizes were compared. Finally, we meshed the volume, which we were highly interested, as extra finer mesh size with cubic elements having sides less than 50 µm and the remaining domains with the fine mesh size. Time step for the calculation is another key factor in the model. The time steps with 1 s, 100 ms, 10 ms, and 1 ms were tested in the model. The large time steps make the simulation value unstable, changing erratically over the time, and the small time steps cost a long time to get the results. To balance the accuracy of the simulation results and the time to calculation, we choose time step as 10 ms for the second pulse length simulation.

Another part of the model we could improve in the future is to let the geometry include the electrodes and the leads of the MEA. The present model might be underestimating the temperature rise at the cell layer since the laser energy would be highly absorbed by the ignored electrodes and leads.

7.2 Neural Response

INI has been researched for a variety of application in vivo, such as the peripheral nervous system [47-49, 51], the cochlea [54], the central nervous
system [12, 36], and the heart [68], and also for the application in vitro [89]. Our results also demonstrated the inhibition of neural network activity in vitro with infrared light, as well as the potential utility of this technique on some certain types of neurological disorder diseases. So far the primary mechanism of both INS and INI are believed to be thermally mediated. INS is believed to be a consequence of the spatiotemporal temperature gradient (dT/dt and dT/dx) [28, 29] and the INI is a result of the temperature rise (ΔT) [51]. Duke et al. [51] have reported that inhibition in vivo is a function of a relative increase in baseline temperature (ΔT). They found that the temperature rise required for a propagation block was approximately 8 °C in unmyelinated buccal nerve 2 (BN2) of Aplysia and 9 °C in the myelinated rat sciatic nerve of the rat. The degree of the INI is increased with the temperature rise in vivo with infrared light, as well as the potential utility of this technique on some certain types of neurological disorder diseases. So far the primary mechanism of both INS and INI are believed to be thermally mediated. INS is believed to be a consequence of the spatiotemporal temperature gradient (dT/dt and dT/dx) [28, 29] and the INI is a result of the temperature rise (ΔT) [51]. Duke et al. [51] have reported that inhibition in vivo is a function of a relative increase in baseline temperature (ΔT). They found that the temperature rise required for a propagation block was approximately 8 °C in unmyelinated buccal nerve 2 (BN2) of Aplysia and 9 °C in the myelinated rat sciatic nerve of the rat. The degree of the INI is increased with the temperature rise in paper II implies that the mechanism of INI is related to the temperature rise.

Two different baseline temperatures were used in our experiments. In Paper I, the baseline temperature of 32 °C was used. The maximum temperature increase is only about 5 °C and we reach a modest maximum temperature of 37 °C. The mean maximum inhibition of 63 % and 88 %, spike data from all electrodes, were obtained for the respective MEAs. In Paper II, a physiological temperature of 37 °C was used as the baseline temperature. The obvious inhibition was found for a temperature rise around 5 to 9 °C, corresponding to the absolute temperature 42 to 46 °C. At this temperature range, most neurons are unequally inhibited from the 10% to 80%. Therefore, our experimental results suggested that a relative temperature rise was involved in the inhibition more than an absolute temperature threshold. The results based on the physiological temperature are more meaningful for translating the findings into clinical applications. However, the global temperature (baseline temperature of the experiment) also plays an important role in INI as seen from previous simulation studies [50, 83]. Mou et al. [50] reported that a different local temperature rise (ΔT) was required for the inhibition at different global temperatures. The required temperature rise is smaller when the global temperature is higher. The question of what the relationship between local temperature rise and global temperature cannot be answered until systematic researches based on the more different baseline temperatures have been done in the future. This would be beneficial to transfer the results from the experimental animals, which have the different physiological temperatures, to the clinical application of the human.

Another interesting aspect of INI is that the several cellular mechanisms of inhibition have been presented, although less than the number of explanations of cellular mechanisms proposed for the INS. Cayce et al. [36] noticed that INI was related to the large beam size (diameter 400 μm), which may evoke the response of the inhibitory neurons. Feng et al. [78] suggested the alteration of GABAergic neurotransmission involved in the inhibition of the neural network.
Wang et al. [68] observed an increased intracellular calcium after the inhibition of the embryonic hearts. It is possible the INI also share some common cellular phenomenon to the INS. A well-known general explanation of the INS is that a rapidly local temperature rise induces an increase of the membrane capacitance, causing a depolarizing membrane current. Albert et al. [73] demonstrated that transient receptor potential vanilloid 4 (TRPV4), a temperature sensitive gated channel, is responsible for the IR-thermal activation of the sensory neurons. All these interpretations illustrate the complexity of the INM. In our limited study, it is impossible to fully understand the mechanism of INI. But in Paper II, the results implied that the inhibitory neurons are not necessary for the inhibition and we also highlighted that potassium conductance change may play a key role in the inhibition of cortex neurons. A prominent increase appeared at the end phase of the action potential during the IR exposure (Figure 21), which implied that more outward potassium current appeared during the laser irradiation based on the theory of the formation of the AP (Figure 5). The network activities could be suppressed when the inhibitory synapses were blocked (Figure 22), which suggested a thermal block contributed to the inhibition of the neurons while we couldn’t exclude activation phenomena of the inhibitory neurons. More details were discussed in the Paper II.
8 Conclusion

The overall objective of this research project was to improve the understanding of the possible mechanism in details and thermal effects of the INM and facilitate the transfer of this innovative modulation technique to the clinical application. The main conclusions from the thesis are summarized below:

- The activity of the cortex neural network can be inhibited by IR and the different degree of inhibition can be achieved by tuning the laser power (Paper I).

- The temperature model provided the guidance to choose the safe laser parameters for the neural modulation experiment and helped to shed light on the underlying mechanism of INI (Paper II).

- The efficiency and safety of INI to modulate the neuronal network activity in vitro can be achieved by increasing the temperature by 5 to 9 °C, above 37 °C (Paper II).

- Laser power increasing has the different impacts on the spike amplitude and spike width, which suggested the efficiency of INI correlating with the action potential amplitude rather than its width (Paper II).

- The induced hyperexcited activity was inhibited by INI suggested that the technique of INI could be potentially applied on some certain type of the neurological disorder diseases and INI can be accomplished without inhibitory synapses (Paper II).

- The inhibition was obtained based on the two different baseline temperatures, which suggests that the relative temperature rise is involved in the INI rather than an absolute temperature threshold (Paper I and Paper II).
9 Future work

Several previous studies in the peripheral nervous system (PNS) have shown the promising ability of INM to be used as a clinical tool for intraoperative nerve monitoring and cardiac pacing, and neural prosthetics, such as cochlear implants. In the central nervous system (CNS), the technique of INM also has been suggested to be employed for the cortical mapping and deep brain stimulation applications. Our primary study of INI also shows the potential of the method to be used as a tool to treat some certain neurological diseases which are characterized by the hyperexcitation of the central nervous system, such as epilepsy. In my opinion, the future of the technique of INM is bright. However, there is still a long way to go before we can transfer the work in the laboratory to the practical application in the life since there are still several questions about this technique we cannot answer. To my knowledge, there are at least five questions need to be answered in the future before the INM can be used as a clinical device in the CNS.

- How will the INM impact on the different subtype cells in the CNS?

As we stated in the background section of this thesis, the CNS is made up of different neurons, such as excitatory and inhibitory neurons, and different glial cells, such as astrocytes, microglia cells, and oligodendrocytes. From our observation, it seems that INM has the different impacts on different cell subtypes. However, the details of these differences have not been investigated. It is important to characterize how INM influence glial cells as they may in turn influence the neural network of the brain.

- What is the fundamental mechanism of the INM?

The previous studies showed the complexity of the cellular mechanisms of INM. These mechanisms are related to the heat-sensitive ion channels (TRPV and TREK channels etc.), intracellular process (intracellular calcium transients), membrane capacitance, and neurotransmitter release. A clarification of these mechanisms needs to be done to understand the effects when using INM on a complex network such as the brain.

- What kind of the laser parameters would be chosen for the application of the INM in the CNS?

The other important question we need answer is what laser parameters are efficient for the specific application. The parametric studies are still few in the CNS compared to the studies in the PNS. Future work should aim at acquiring
more data on laser parameters for the use of INM in the CNS since the CNS is more complicated than the PNS.

- Can INM really cure the patients?

All the results about INM are actually based on the research in the lab, except for two clinical studies. One is for the human ear and another is for the human spinal roots. There is no clinical study in the CNS of the human. In my opinion, clinical trials need to be done before an answer to this question can be found.

- How should we design the medical devices system of the INM

One of the interesting things is that almost all the researches always talk about one of the advantages of the INM is non-invasive, which do not need the physical contact between the electrodes and the neural targets. However, almost all of the animal studies of INM are invasive, which are used in different kinds of surgery to insert the optical fiber into the targets. Thus, I believe that the question of how the light energy could be delivered to the targets to realize a real non-invasive device would be a challenge.
10 References


