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博士論文 (要約)

High Resolution Spatiotemporal Optogenetic Spinal Cord Stimulation

(高時空間分解能光遺伝学的脊髄刺激)

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High Resolution Spatiotemporal Optogenetic Spinal Cord Stimulation

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by

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Abstract

Spinal cord stimulation has long been used as a medical therapy to relieve neuropathic pain. It has also been studied as a research tool to treat motor dysfunction after spinal cord injury. The standard stimulation method involves the placement of electrodes on the epidural spinal cord and conduction of electrical current through them in order to change the neuronal activity. However, in the absence of a definitive cell-type selection and spatial resolution, it is difficult to determine the stimulation target, thereby rendering the underlying mechanism ambiguous.

To overcome these limitations, we aimed to focus on the development of a novel spinal cord stimulation system with high resolution and selectivity that can specify the stimulation target in rat models. Here, we propose to utilize optogenetics to design a spinal cord stimulation mechanism with high cell-type selectivity as well as a multichannel optical stimulation device that has a high spatial resolution.

As a realization of this concept, a Single Laser to Multiple Optical Fiber (SLMOF) device with 720 channels was developed. This system could provide a stimulation resolution 26.7 times higher than the previous research of electrical stimulation. By precisely controlling the projection of laser within the device, we can provide stable light outputs at a low cost.

In order to examine the details of light penetration and absorption on the spinal cord, we simulated the light propagation in a 3D spinal cord model by using a Monte Carlo-based light transport model. The simulation results revealed that a major portion of the light is absorbed in the vicinity of the surface of the spinal cord with some fraction spreading over the surrounding tissue. The data were also verified by *in-vitro* assessment of light penetration within spinal cord tissue slices. Compared to electrical stimulation, which affects the neural activity in a broad region of the spinal cord, light strictly propagated in a restricted area within the nervous structure.

W-TChR2V4 transgenic rats that express the light-gated ion channel in mechanoreceptive and proprioceptive neurons of the spinal cord were used to validate the results. We also set up a spinal cord injury model for demonstrating the application of our device. In order to interface the optics to the animal model, we designed a

novel spinal cord window.

Then we carried out a series of *in-vivo* experiments to evaluate our method and compare it with the existing electrical stimulation method. First, we confirmed that the optogenetic spinal cord stimulation could induce paralyzed muscle reaction after spinal cord injury. Then, we measured the EMG response latency of each stimulation, revealing that our epidural optical stimulation only directly affected the afferent neurons and interneurons on the dorsal part but not motoneurons. Also, analyzing the muscle-contraction-related stimulation spots on the epidural spinal cord indicated the capability of our system to stimulate different somatosensory pathways with greater selectivity in comparison to that of electrical stimulation.

Through this research, we demonstrated that our multichannel optogenetic spinal cord stimulation system can stimulate the somatosensory pathways in the spinal cord in rat models with a higher resolution and selectivity than in the case of an ordinary electrical stimulation setup. We plan to use the system to study how epidural stimulation modulates the functional output of the spinal cord during spinal cord injury. We believe that adopting this system would aid in our comprehension of the mechanisms underlying specific spinal cord responses during therapy post injury.

Acknowledgment

This thesis would not have been accomplished without the help of many people. First, I would like to express my sincere gratitude to my advisor Professor Yasuo Kuniyoshi. He taught me the way to think broadly and deeply and encouraged me to challenge everything that is valuable for understanding the truth of intelligence. Besides my advisor, I would like to thank Professor Hiroshi Onodera who inspired me with the idea of optogenetics, helped me organize the entire research plan and gave me much precious advice about neuroscience and animal experimental design.

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Chapter 1

Introduction

Spinal cord stimulation (SCS) is a technique that artificially changes the activity of spinal cord neural network. It has been utilized as a tool in medical treatment or research for patients with intractable pain, spasticity and spinal cord injury (SCI). However, due to its non-selective stimulation of cellular activities, the detailed mechanisms underlying it remain unclear and its effectiveness as a medical treatment has displayed disparate results between subjects.

Here, we first describe the research motivation behind developing a new spinal cord stimulation system and the significance of improving the stimulation resolution. Subsequently, we define the research goals and the scope of this thesis.

1.1 Background

1.1.1 Development and Application of the Spinal Cord Stimulation

SCS was initially developed to treat chronic neuropathic pain. Melzack and Wall proposed the Gate Control Theory of pain in 1965 [55]. They speculated that stimulation of large non-nociceptive myelinated fibers ($A\beta$ fibers) of the peripheral nerves could inhibit the activity of small nociceptive fibers ($A\delta$ and C fibers). Based on this theory, Shealy *et al.*, conducted the first medical attempt to insert a stimulator into the dorsal column of patients suffering from cancer pain [82].

Thereafter, the technique of SCS has been greatly advanced and the medical use of SCS to relieve chronic neuropathic pain has been approved in many countries including Japan [96]. The advantages of reversibility and safety compared to ablative approaches endorses SCS as the recommended therapy to treat intractable pain of diverse origins such as spinal cord injury, ischemia of the extremities, angina, and cancer [18, 41, 86].

The use of SCS for treatments other than pain control initiated from a serendipitous diagnostic outcome. A patient with multiple sclerosis who was undergoing SCS treatment to alleviate the pain was reported to have improved motor function [9]. Since then, research on treating motor dysfunction with SCS has considerably increased.

Spinal cord injury in the cervical or thoracic region may cause long-term reduction of segmental inhibition and excessive velocity-dependent muscle contraction, which results in a symptom called spasticity [1, 20, 35]. SCS was employed by a number of groups to treat such spasticity [4, 74, 75]. However, limited understanding of the pathophysiology of such spasticity cases caused the use of SCS to be stagnant in experimental status [65, 86].

During the examination of suitable stimulation parameters for spasticity control in patients with spinal cord injury, SCS-induced rhythmic muscle activity in paralyzed legs was repeatedly observed [17]. Since the local neural circuits, responsible for sensory-motor integration, remain intact in most spinal cord injury cases, it is considered that SCS could reactivate these circuits to generate motor outputs.

Further, studies on SCS to reactivate paralyzed motor function indicated that applying tonic stimulation at 25-50 Hz on the T11-12 vertebral segments can elicit locomotor-like activity in paraplegic patients [25, 26]. Recent investigations also report that patients diagnosed as completely paraplegic can perform independent stepping after long term task-specific training in the presence of SCS [30, 89]. These findings make SCS one of the most promising method to restore the deficit motor function in spinal cord-injury patients.

1.1.2 Limitations of Spinal Cord Stimulation and Demands for High Resolution Stimulation

Albeit spinal cord stimulation is used as a medical treatment to relieve neuropathic pain and has shown the potential to restore motor dysfunction after spinal cord injury, the detailed mechanism underlying it remains unclear. Moreover, many patients were reported failures of SCS treatment for unknown reasons, have been documented [13, 21, 80, 86].

The general way of administering spinal cord stimulation is by implanting the electrodes onto the epidural spinal cord. The area of influence depends on the orientation of the cathode and the anode. The electrical current induces depolarization in neurons and changes the activity of spinal cord circuits. As described previously, the main target of SCS was supposed to be the large myelinated fibers of the peripheral nerves ($A\beta$ fibers) based on the Gate Control Theory [55]. However, due to the non-selectivity of electrical stimulation and the high conductivity of biological

tissues, it is almost impossible to specify the stimulation target by current electrical stimulation techniques [11].

Recent studies have shown that alternative mechanisms might play a more important role in SCS-mediated alleviation of neuropathic pain. In the animal model, the release of neurotransmitters involved in pain modulation in the spinal cord, such as gamma-aminobutyric acid (GABA), substance-P and serotonin was elevated upon SCS [51, 84]. These neurotransmitters are known to suppress the hyperexcitability of wide dynamic range (WDR) neurons in the dorsal horn and attenuate the pain condition [71, 56, 94].

The prevailing idea on the mechanism of SCS-triggered reactivation of motor dysfunctions during spinal cord injury is that epidural electrical stimulation (EES) mainly targets the dorsal roots and dorsal ascending spinal columns [8, 70]. The activated neurons then send the signals to various spinal reflex circuits [58, 63, 78], circuits involved in the regulation of sensory and motor information [36, 67], and rhythm and pattern generating neural networks [12, 60, 57] to contribute to motor outputs [37]. However, there was no evidence that showed a direct effect of electrical stimulation on motor neurons [28, 50]. Moreover, the activated sensory pathways from different muscles largely overlapped with another [24]. Thus, the complex interaction between different neural circuits to produce a motor output remains unclear.

In order to improve the effectiveness of SCS in medical use, a better understanding of the mechanism underlying it is necessary. However, current electrical stimulation technique has its limitation on specifying the stimulation target. There is a demand for developing new spinal cord stimulation technique with higher selectivity on the stimulation target.

1.2 Objective

1.2.1 Purpose of the Thesis

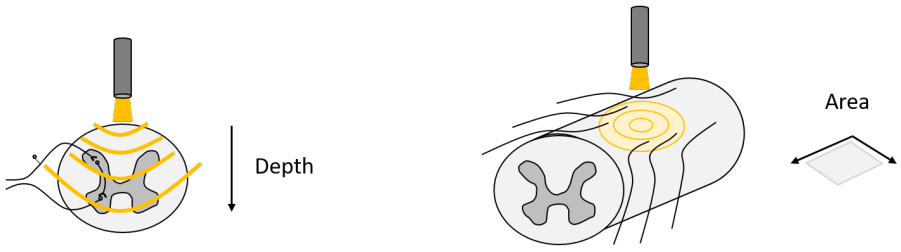
The objective of this thesis is to develop a high-resolution SCS. We aim to restrict the stimulation target on the dorsal surface of the spinal cord with fewer neural circuits being activated than the ordinary spinal cord stimulation method.

1.2.2 Scope of the Thesis

The designed system is supposed to be used as an tool for understanding the mechanism of SCS in the modulation of spinal cord neural activity. As a tool for neuroscience research, the experimental subject we used in this thesis is the rat animal

model.

As described previously, there are numerous disorders wherein SCS could be used as a therapy. We focused on the function of SCS to modulate motor signal outputs in spinal cord injury condition in this thesis. It has been shown that the SCS modulates the motor outputs mainly by affecting the proprioception pathway [22, 62, 85], and the spatiotemporal parameters are important for producing functional outputs [92, 89]. However, it is difficult to discriminate whether the motor signal output induced by ordinary SCS method is a result of direct stimulation effects or indirect interactions within the complex spinal cord neural circuits. In this thesis, for specifying the target of our stimulation, we define and discuss the stimulation resolution in two aspects: the depth and the area of threshold photic neuronal stimulation (Figure 1.1).



(a) The depth of threshold photic neuronal stimulation

(b) The area of threshold photic neuronal stimulation

Figure 1.1: The resolution of a stimulation applied from the dorsal part of spinal cord was evaluated by (a) the depth from the dorsal surface and (b) the area on the spinal cord surface.

- The depth of threshold photic neuronal stimulation:

  From the dorsal to the ventral side of the spinal cord, the neurons in a sensory-motor pathway can be separated into sensory neurons, interneurons, and motor neurons. The mechanism of SCS to produce functional motor outputs is through the stimulation of sensory neurons and interneurons. However, ordinary SCS also has direct effects on motor neurons [28, 50].

  In this thesis, we tried to restrict our stimulation on the dorsal surface of the spinal cord. Hence, there should not be any direct stimulation effects on the motor neurons on the ventral side.

- The area of threshold photic neuronal stimulation:

Sensory nerves from different muscles traverse through the dorsal root ganglion and enter the spinal cord on the dorsal side. The entry sites of these nerves have specific distribution on the spinal cord dorsal area. Previous SCS research targeted the stimulation on the whole area of each segmental, which induced the activities of several somatosensory pathways from different muscles.

In this thesis, we tried to make the area of influence as small as possible. In this way, we supposed fewer sensory nerves to be activated during each stimulation. Thus, a more exclusive neural stimulation could be achieved.

1.3 Structure of the Thesis

In order to achieve high-resolution SCS, we discuss the possible approaches, system design, and evaluate their performance in the subsequent sections. The overall structure of this thesis is illustrated in Figure 1.2.

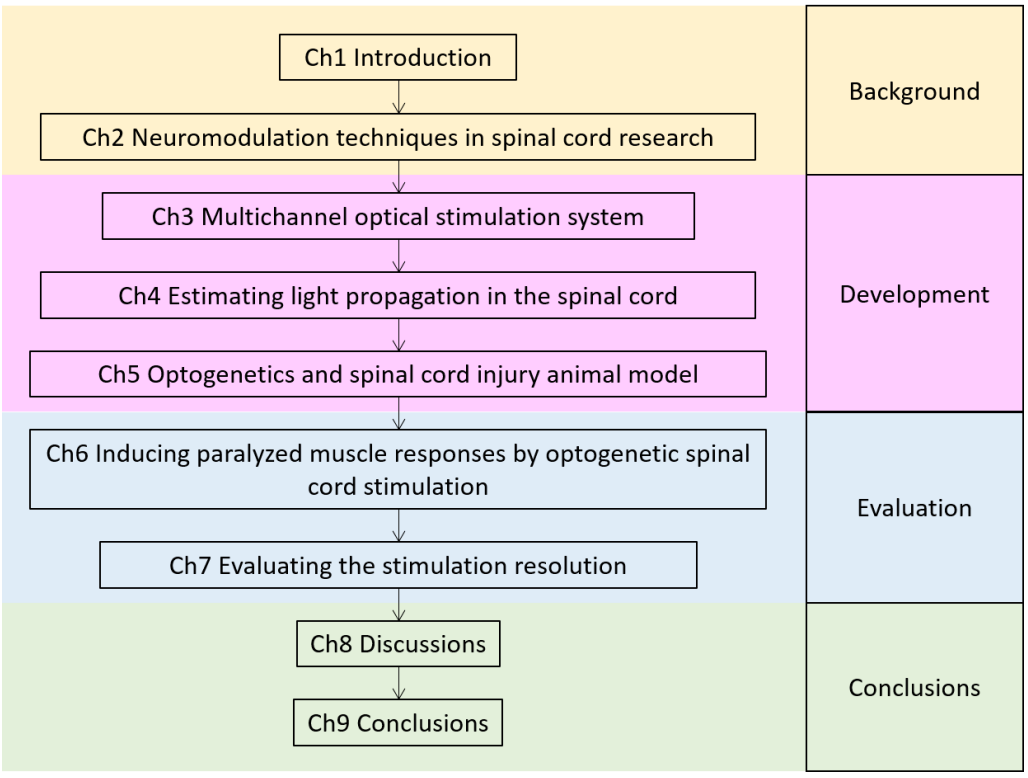


Figure 1.2: Structure of the thesis.

- Background:

  First, we discuss the importance and the possible approach to achieve high-resolution spinal cord stimulation. In the following chapter, we peruse available literature on neuromodulation techniques used nowadays along with the challenges associated with the stimulation of the spinal cord. Then we propose a novel approach to achieve high-resolution spinal cord stimulation by utilizing optogenetics and multichannel laser optics technique.

- Development:

  Next, we describe the setup of the proposed system. In chapter 3, we develop the Single Laser to Multiple Optical Fiber system and test the performance of its optical output. In chapter 4, we evaluate the feasibility of our proposed system by using computer simulation to test the depth and area of influence of the stimulation. In chapter 5, we elaborate on the animal model used in the study, including information about animal species, transgenic line of optogenetic, surgery procedure and animal care.

- Evaluation:

  After the system is developed, we evaluate the system's capability of stimulating the spinal cord neurons *in-vivo*. In chapter 6, we test the capability of optogenetic spinal cord stimulation to induce paralyzed muscle reaction and exclude the side effects that might affect the motor output during light stimulation. In chapter 7, we evaluate the stimulation resolution of our system and compare it with the ordinary electrical stimulation results.

- Conclusions:

  Finally, in chapter 8 and chapter 9, we summarize the results into conclusions, explain the contribution of this research and discuss its future prospects.

Chapter 2

Neuromodulation Techniques for Spinal Cord Research

Toward our research goal, i.e., "developing a high-resolution spinal cord stimulation system," we discuss and propose our concept to design the system in this chapter.

We first survey the state-of-the-art technology for modulating neural activity. Then, we describe the structure of the spinal cord and the challenge associated with stimulating it. Upon weighing the pros and cons of different methods, we proposed our novel spinal cord stimulation design that combines optogenetics and multichannel laser optics technique for high-resolution neural stimulation.

2.1 Neuromodulation Techniques

2.1.1 Mechanism of the Neuromodulation

The nervous system transmits signals between neurons. This process includes electrical and chemical responses.

The phospholipid bilayer of neuronal cell membranes blocks the diffusion of materials and ions. Ions are only allowed to be transferred across specific channels on the membrane, which results in different voltage states across it. In the resting state, the intracellular potential is usually 60 to 80 mV lower than the extracellular. When stimuli depolarize the membrane potential to a threshold level (about -50 to -55 mV), an action potential would be created and the membrane potential would be reverted to a positive value (about +40 mV) by voltage-gated sodium channels. This action potential would quickly spread across the membrane. When the electric signal reaches the end of an axon, chemical compounds called neurotransmitters are released. These molecules would bind to the receptors of the other neurons and create new action potential for signal transmission.

Neuromodulation works by changing the state of neurons at any points during a signal transmission process. It can either excite or inhibit the transmission to control the output of a neural circuit.

2.1.2 Electrical Stimulation

The finding that direct electrical stimulation could change neural activity can be traced back to the experiments conducted by Luigi Galvani in the 18th century. He found that an electrical spark can induce twitches in the muscles of dead frogs. These findings opened the research field of bioelectricity.

We now know that a potential gradient applied by electrodes can cause depolarization on the cell membrane of neurons which results in neural stimulation. A different potential gradient can be applied to cause a regional hyperpolarization, which blocks the propagation of action potentials and results in neural inhibition. A more complex control of neural activity is possible by using different waveforms of electrical current that exploit the time constants of different ion channels [53].

In general, electrical stimulation has a high temporal resolution to control the timing and frequency of generating the action potential. However, due to the conductivity of biological tissue, it has low spatial resolution and no cell-type specificity [11]. Depending on the arrangement of electrodes, the stimulation might affect a large area of nerves and muscles around it which results in an unpleasant and painful experience for the subjects.

Electrical stimulation is suitable for utilization in large scale, unfocused neural activity promotion or suppression. Clinically, it has been used in Deep Brain Stimulation to treat movement disorders such as Parkinson's disease [33, 46], and in SCS to relieve chronic neuropathic pain [18, 41, 86]. In neuroscience research, it also shows the potential to restore the deficit motor function after spinal cord injury [25, 26, 30, 88, 89].

2.1.3 Magnetic stimulation

The other neuromodulation technique, magnetic stimulation, works similar to the electrical stimulation. By rapidly changing the magnetic field, an electrical current can be produced and the action potential on cell membrane can be induced.

The advantage of using magnetic stimulation is that the magnetic field can be produced remotely from the coil. As a result, the action potential of neurons can be induced by non-invasive methods and the unpleasant and painful feeling of stimulation could be largely reduced. This principle has been used as transcranial magnetic stimulation (TMS) in clinical use to treat stroke and depression [76].

However, TMS devices usually have a large power consumption and have even lower spatial resolution than electrical stimulation. Furthermore, the magnetic field produced by TMS devices may heat the other metal materials, so it cannot be used on patients who have been implanted with other electronic devices like the pacemaker.

2.1.4 Thermal stimulation

A temperature change in the tissue environment can change the physiological state of cells. This may affect the transmembrane capacitance and the conductance dynamics of ion channels, which result in a change in the membrane potential of neurons.

Several mechanisms have been developed to modulate neural activity by thermal stimulation. These methods include use of near infrared laser to directly heat the target neurons [19, 91] or employment of nanoparticles to absorb microwave and heating the tissue around them indirectly [38].

These thermal stimulation methods can be controlled in a precise area, contributing to high spatial resolution. However, they have a low temporal resolution for repeated stimulation. Also, tissue damage due to heat accumulation is an issue with such a technique. Currently, thermal stimulation is mainly used in scientific research.

2.1.5 Chemical stimulation

The traditional pharmacological approach can also be used to modulate neural activity. It works either by changing the ion concentration around neurons to suppress or promote action potential [83] or by introducing agonist or antagonist against specific neurotransmitters to affect signal transmission [87, 88].

However, due to the dynamics of chemical diffusion and the bioavailability of the molecules within the tissue, it is very difficult for chemical stimulation to achieve high spatial and temporal resolution. On the other hand, this approach is suitable for conditions that need long term and large area stimulation, such as deep brain stimulation [53].

2.1.6 Optogenetic stimulation

For selectively inducing a specific cell's activity, a neuromodulation technique called optogenetics has been developed [5]. Optogenetics combine the techniques from optics and genetics to control specific cell activity in live tissues [5, 16, 14, 15, 79].

There are some light-gated ion channels that can absorb a specific wavelength of light and open the channel to depolarize the cell (Figure 2.1). Channelrhodopsin-2 (ChR2), which reacts to blue light, is a leading example found in green algae. Except for retina cells that have photoreceptor proteins, animal tissues do not have these type of channels and hence that they do not react to light exposure. However, by genetically modifying the cells to express the light-sensitive ion channels on it, they can be depolarized by light (with specific wavelength) exposure.

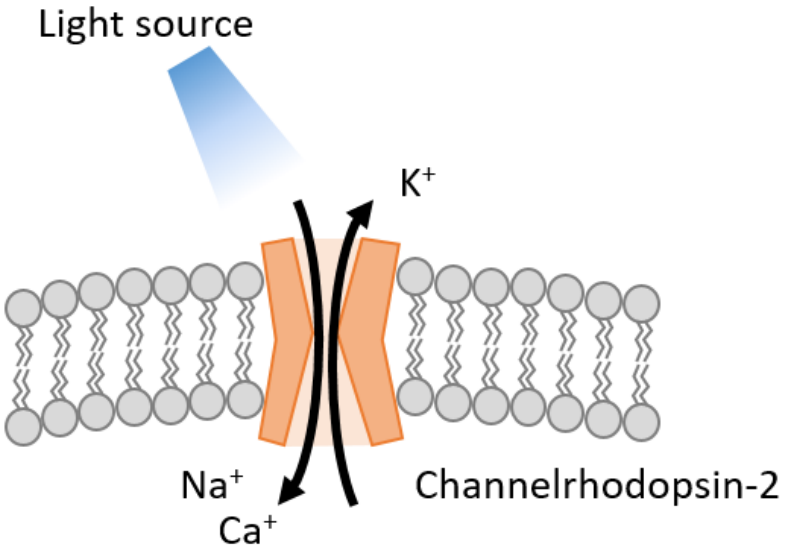


Figure 2.1: Mechanism of the light-gated Channelrhodopsin-2.

Optogenetics has advantages of high spatial and temporal resolution. Using genetic engineering technique to express light-gated ion channels in cells with specific selection marker would also contribute to cell type selectivity. On the other hand, the process of genetic manipulation raises the issue of safety and ethical concerns over the use of the technique on human subjects. Currently, the applications of optogenetics are limited to scientific research.

2.1.7 Comparison between different neuromodulation techniques

Although the properties of different neuromodulation approaches may be changed by different devices and methods, the standard features of neuromodulation techniques nowadays can be outlined in Table 2.1. Among these techniques, only optogenetic can select the type of cells to be activated or inhibited with optimal spatial and

temporal resolution. This advantage make optogenetic a strong tool in neuroscience research.

Table 2.1: Comparison between different neuromodulation techniques [53].

Properties	Electrical	Magnetic	Thermal	Chemical	Optogenetic
Spatial resolution	$10 \sim 100\,\mu m$	$100\,\mu m \sim 1\,cm$	Single cell $\sim 10\,\mu m$	Depends on diffusions in tissue	Sub-cellular
Cell type specificity	No	No	No	No	Yes
Temporal resolution	≤ 1 ms	$1 \sim 10$ ms	1 ms $\sim$ 10 s	1 s $\sim$ 1 min	$1 \sim 10$ ms
Invasiveness	Either	Either	Yes	Yes	Either
Gene manipulation	No	No	Either	No	Yes
Health risk	Electro-chemical reaction	Stroke, eddy current	Thermal tissue damage	Electro-chemical reaction	Phototoxicity
Application on human	Yes	Yes	No	No	No

2.2 Spinal Cord

2.2.1 Structure of the Spinal Cord

The spinal cord is a long tubular structure made up of nervous tissue. It is the information pathway that connects the brain to the peripheral body. Neurons in the spinal cord can be separated into three groups:

- Afferent (sensory) neurons.
- Interneurons.
- Efferent (motor) neurons.

The main functions of the spinal cord include nerve signal transmission between the central nervous system (CNS) and the peripheral nervous system (PNS), its activity as the coordinating center of spinal reflex [54], and special neural circuits called central pattern generators (CPG) that generate rhythmic outputs, independent of brain control [12, 17, 32].

When observed in the cross section (Figure 2.2), the center of the spinal cord is a butterfly-shaped grey matter, which consists of cell bodies of interneurons, motor neurons, neuroglia cells and unmyelinated axons. On its outside, there is the white matter, which consists of the myelinated motor and sensory axons. These nervous tissues are enclosed in and protected by the bony vertebral column (spine).

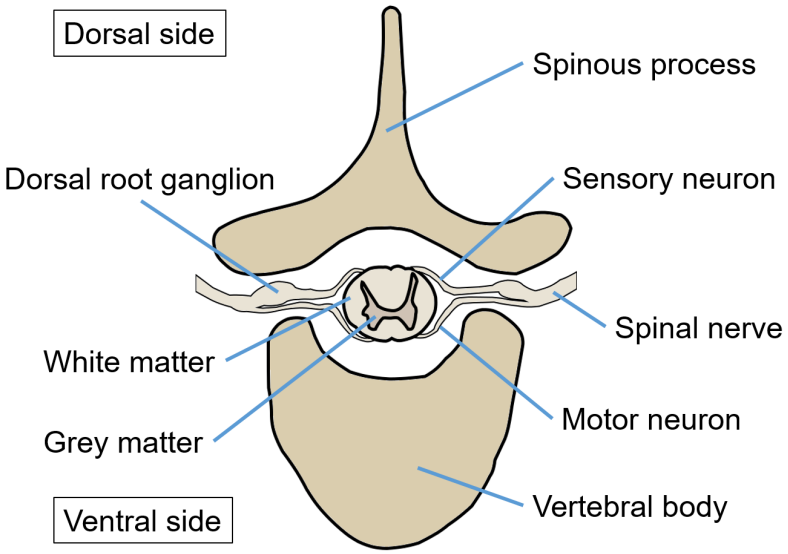


Figure 2.2: Cross-section of the human spinal cord [66].

Based on the cellular structure and the function, Bror Rexed [72, 73] labeled the grey matter into 10 laminae (layers). The distribution of these laminae is slightly changed between different segments. The Rexed laminae of the rat spinal cord segment L2 is plotted in Figure 2.3.

Afferent neurons, which carry sensory information from muscles and skin, enter the spinal cord through the dorsal spinal nerve roots and innervate the region of lamina I to V. In contrast, efferent neurons, which innervate the muscles, have their nuclei in lamina IX and leave the spinal cord through the ventral roots [29, 61, 90]. These dorsal and ventral roots merge together and form the spinal nerves on the lateral side of the spine.

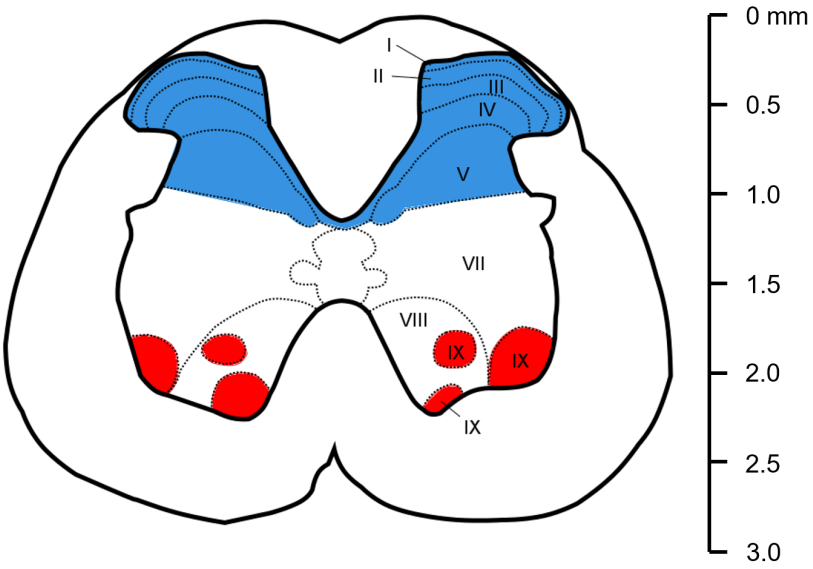


Figure 2.3: Rexed laminae of the rat spinal cord segment L2. The region of lamina I to V (blue) contains the axons of sensory neurons, and the lamina IX (red) corresponds to the area that the nuclei of motor neurons are located [61, 90].

There are 31 spinal cord nerve segments in humans (35 in rat). According to the body area that they innervate, they can be separated into 5 groups (Table 2.2). Since the length of the spinal cord is shorter than the length of the spine, in an adult human, only the cervical segments are at a level position with their corresponding vertebrae. Below the cervical level, spinal nerves entering the vertebral column would travel vertically for a small distance before entering the spinal cord (Figure 2.4). The T12 segment is in level with the T9-T10 vertebra, the L5 segment is in level with T11-T12 vertebra, and the spinal cord ends at L1-L2 vertebra [68, 69].

2.2.2 Spinal Cord Injury

Damage to the spinal cord can cause permanent impairment of its function. A variety of symptoms may show up, depending on the severity and the segmental location of the injury.

Spinal cord injury (SCI) can be incomplete, when it still retains some control on signal transmission in the neural pathway or complete, when it totally loses its function of sensation and muscle control. General classification is based on the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) (Table 2.3), published by the American Spinal Injury Association (ASIA).

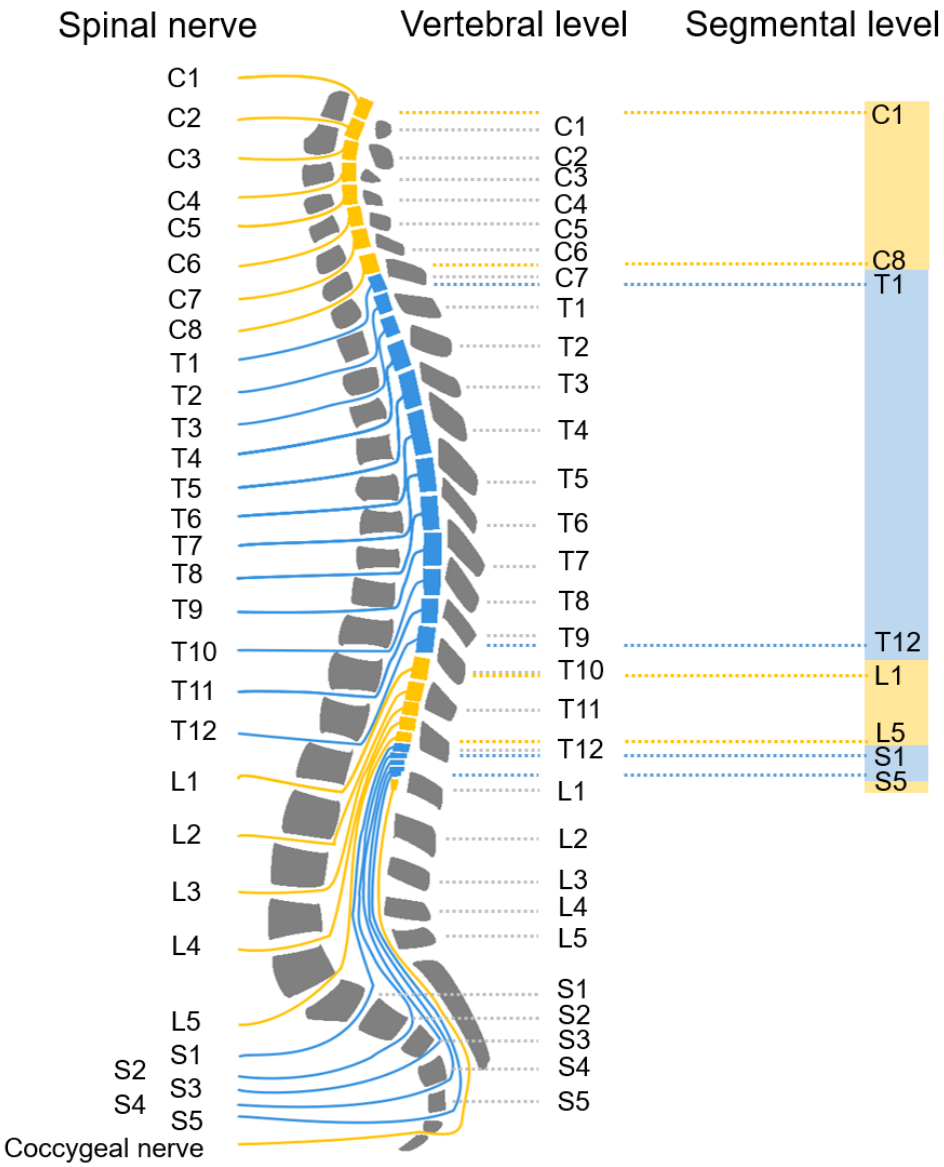


Figure 2.4: Relative positions of vertebral levels and segmental levels in human spinal cord [68].

Table 2.2: Spinal cord segments in human and rat [81].

Segments	Human	Rat
Cervical nerves	8	8
Thoracic nerves	12	13
Lumbar nerves	5	6
Sacral nerves	5	4
Coccygeal nerves	1	3

Damage to the spinal cord causes functional impairment of body parts corresponding to the innervated spinal nerves at and below the lesion site. SCI at or above the lumbar or sacral segments of the spinal cord may cause functional impairment in the legs, hips and genitourinary system. Further, damage to the thoracic spinal nerves may cause muscle dysfunction in the trunk. Most of all, if SCI occurs at the cervical level, it could result in tetraplegia or even breathing dysfunction.

Many reasons can be attributed as the cause of SCI including physical trauma, infection, insufficient blood flow, and tumors. Amongst them, motor vehicle accidents, falls, violence and sports injuries are the most common causes of SCI in the United States of America [77]. It has been estimated that about 10.4 to 83 new cases of SCI occur per million population every year. [93].

2.2.3 Treatment for Spinal Cord Injury

The research on SCI treatment can be mainly classified into two types: Direct repair of the neural pathway or bypassing the lesion to control the body. Efforts on repairing the neural pathway include neuroprotection, which is to prevent the harm that ensues from a secondary injury to the spinal cord, and neuroregeneration, which aims to reconnect the broken neural pathway [3, 43]. With the breakthrough in stem cell research, replacing damaged spinal cord cells with stem cell transplantation is thought to be a promising prospect in the cure of SCI.

The approach of bypassing the lesion to control the body, on the other hand, does not directly cure the injured neural tissue. It essentially employs assistive devices to artificially control the spinalized body parts. One of the examples of this approach is the robotic exoskeleton, which is a wearable mobile machine that is powered by actuators, such as electric motors, to support paralyzed body motion.

Table 2.3: The International Standards for Neurological Classification of Spinal Cord Injury [44].

Grade	Classification	Description
A	Complete injury.	No motor or sensory function is preserved in the sacral segments S4 or S5.
B	Sensory incomplete.	Sensory but not motor function is preserved below the level of injury, including the sacral segments.
C	Motor incomplete.	Motor function is preserved below the level of injury, and more than half of muscles tested below the level of injury have a muscle grade (Table 2.4) less than 3.
D	Motor incomplete.	Motor function is preserved below the level of injury and at least half of the key muscles below the neurological level have a muscle grade (Table 2.4) of 3 or more.
E	Normal.	No motor or sensory deficits, but deficits existed in the past.

Several exoskeletons have been developed such as the ReWalk Robotics exoskeleton and the Ekso Bionics [47, 52] etc. By restoring normal body activities in the paralyzed regions, it prevents muscle weakness and muscle wasting. Furthermore, in combination with active training, a functional shift of the remnant neural pathway to cover the damaged one is expected.

Another bioengineering technique to treat SCI is spinal cord stimulation, which will be discussed in depth in the following section. Even without brain signals, spinal cord neuron activities can be induced by directly applying stimulation such as electrical stimulation. Previous research has shown that long-term rehabilitation training together with epidural electrical stimulation (EES) restored partial ability to stand and step in a clinical case of complete lower-limb paralysis [30].

Despite the advancement in spinal cord research, there is still no proven treatment to completely recover the function of an injured spinal cord . In hospitals, preliminary treatment is focused on basic life support and prevention of further injury. After the condition becomes stable, rehabilitation is initiated to avoid muscular atrophy or other circulatory functional diseases.

Table 2.4: Muscle strength classification [44].

Grade	Muscle function
0	No muscle contraction.
1	Muscle flickers.
2	Full range of motion, gravity eliminated.
3	Full range of motion, against gravity.
4	Full range of motion against resistance.
5	Normal strength.

2.3 Proposed Method to Stimulate Spinal Cord with High Resolution

2.3.1 The Challenges of Modern Spinal Cord Stimulation Methods

As described in the previous section, currently no proven therapy exists that can completely cure SCI. However, the neuronal networks below the injured site remain intact. It has been shown that local spinal cord networks are capable of computing continuous motor processing of proprioceptive and cutaneous input during coordinated motor behaviors like standing and stepping [31]. The networks including spinal reflex and CPG can be activated even without brain control. That paves the way for a patient with SCI to regain control over the silent networks by artificial stimulation of the spinal cord.

The most frequently used neuromodulation technique nowadays is electrical stimulation. Different groups have worked on different techniques of electrical stimulation of the spinal cord and studied their effects on reactivating spinal cord function. However, using electrical stimulation to modulate spinal neurons in a live animal is challenging due to the following reasons.

- The bendable structure of the spine of the animal.
- The highly condensed network of different types of neurons inside the spinal cord.

- No cell-type selectivity and low spatial resolution as described in previous section.

Unlike brain stimulation or stimulating neurons *in-vitro*, *in-vivo*spinal cord stimulation is relatively difficult due to the anatomy structure. Spinal nerves that control the lower limb motion are mainly distributed from lumbar to sacral segments. The joints between the segments are bendable which causes the stimulation device difficult to be mounted at a fixed location and affects the accuracy of stimulation. Currently, most of the SCS researches target the whole segment with very low resolution.

Transcutaneous spinal cord stimulation involved the application of electrical current from the surface of the skin [48, 59]. Although this non-invasive method is relatively safe and simple to execute, it is difficult to restrict the area of influence. In contrast, epidural electrical stimulation directly implanted the electrode onto the surface of the spinal cord. This could limit the effects of electrical current to a smaller area, but the invasive procedure carried the risk of inflicting further damage to the spinal cord tissue.

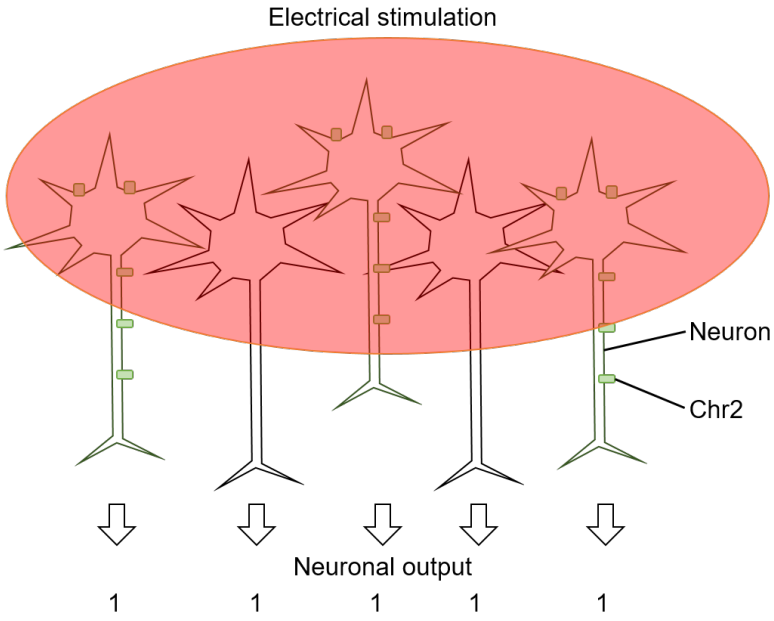
In the animal study, EES elicited rhythmic hindlimb movement in complete spinal cord transection rats when applied to L2 or S1 [10, 27, 34, 39, 40, 49] spinal segments. Similarly, in human patients with SCI, electrical spinal cord stimulation has been shown to activate silent spinal circuits [2, 12, 17, 30, 34].

The effect of EES on activating spinal cord function is known to work through afferent spinal nerves [62, 85]. However, spinal nerves from different parts of the body merge together before entering the spinal cord. Furthermore, the conductivity of the human body and the non-specificity associated with electrical stimulation of distinct cell types [11] make it difficult to distinguish the type of input. Hence, the precise mechanism underlying the effects of EES is hard to determine, thereby restricting the usage of such a stimulation mechanism.

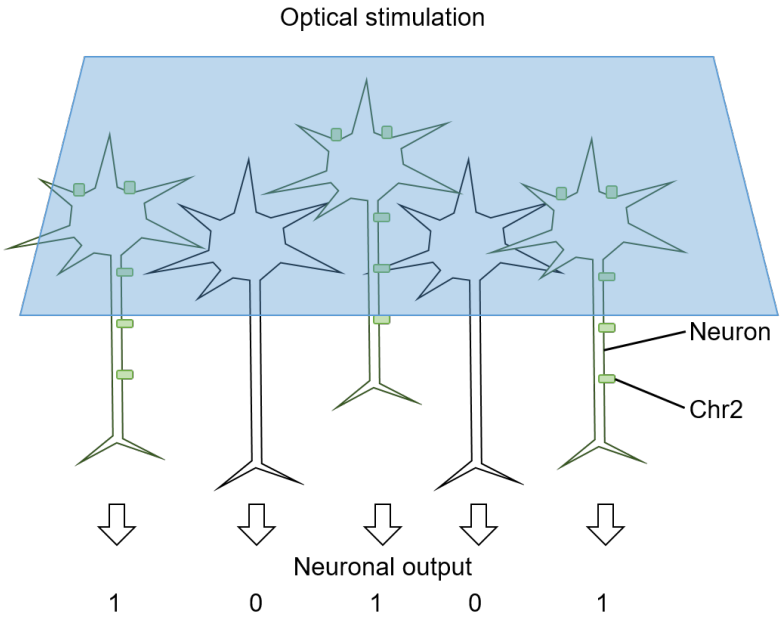
To overcome the challenges faced by modern electrical spinal cord stimulation method, we considered to utilize optogenetics to stimulate the spinal cord. Compared to other neuromodulation methods, the advantages of spatial resolution and cell-type selectivity make optogenetics a powerful tool in studying the function of neuronal networks (Figure 2.5).

2.3.2 The Novel Concept of Optogenetic Spinal Cord Stimulation

As described, the spinal cord contains many important local circuits to control body movement. Electrical stimulation of the epidural spinal cord can aid in the recov-



(a) Electrical stimulation



(b) Optogenetic stimulation

Figure 2.5: Influence of electrical stimulation vs optogenetics. (a) Electrical stimulation affects all cells around. (b) Optogenetics selectively induce target cell's activity.

ery of stepping and standing control, which is lost after spinal cord injury. EES, nowadays, has a very low resolution on the area of influence. In contrast to EES, Optogenetics can selectively stimulate specific neural circuits by expressing opsins in target cells. However, for studying the motion control of the spinal cord, it is necessary to selectively target multiple neural pathways that process information from different muscles at the same time. Presently, there is no appropriate optogenetic animal model that can fit this requirement.

To perform a high-resolution SCS, we propose a novel concept that combines optogenetics with a multichannel stimulation system. We hypothesize that light propagation in the spinal cord can be restricted to a greater degree compared to electrical current spreading through the tissue. The idea is to use optogenetics to target the somatosensory related neural circuits and to select different circuits by the high spatial resolution of multichannel optical stimulation (Figure 2.6).

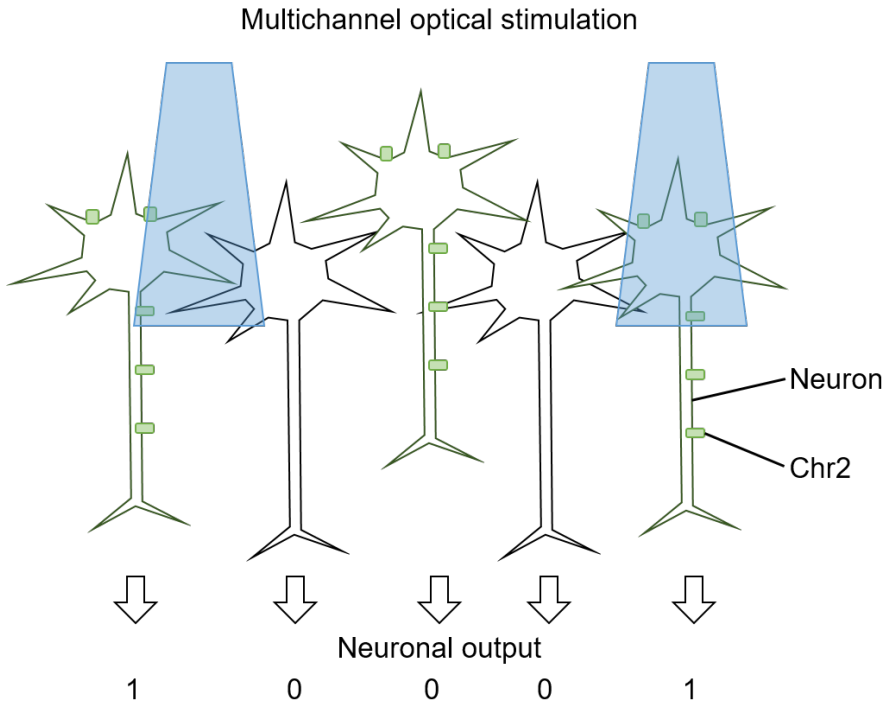


Figure 2.6: The novel concept of spinal cord stimulation that combines the optogenetics and multichannel stimulation system. Optogenetics exclude parts of the non-target cells. Multichannel deliver stimulation to desired neurons that distribute in specific region.

Chapter 3

Development of a Multichannel Optical Stimulation System

In this chapter, we describe the design of the optical stimulation device used in this thesis. In contrast to ordinary optogenetic studies, which used a single light source to stimulate one location at a time, we wanted to build a system that can quickly change the stimulation locations based on the different experimental conditions. Furthermore, the stimulation should have a high resolution on the target spinal cord region in rats that are alive and might move their body to some degree during the experiment. These demands greatly increased the difficulties of the system design.

We examined the requirements specification of the system based on the experimental conditions, our simulation results, and previous research. Then, we proposed a novel design of a multichannel system. After the device was fabricated, we discuss the necessary process to ensure the consistency of output from different channels and the parameters for controlling the device.

Parts of the contents in this chapter have been published in [6, 7]. Reusing the published content in this dissertation is approved by the publisher.

3.1 Requirements Specification

The purpose of the device is to utilize optogenetics on evaluating the effects of spatiotemporal neural activities of specific neuron network function. Our system must achieve the following to be applicable for use in such research.

- Enough light power to activate the light-gated ion channels.
- Spatiotemporally controllable patterns of stimulation.
- Compatibility with in vivo epidural spinal cord stimulation.

- Low occurrence of non-specific effects, e.g. thermal effects.

For developing the system, we were required to consider the distribution of neurons that we were interested in and the light condition that was necessary to evoke ChR2 activity. In this thesis, we especially focused on stimulating the hindlimb-related neural circuits in the epidural spinal cord in rats, which had been introduced Channelrhodopsin-2 (ChR2) into the neurons.

The hindlimb motion-control-related spinal cord epidural area is located from segment levels L2 to S1, which correspond to vertebral levels T13 to L2. This is approximately $3.5 \times 12\,mm^2$ in size, which our device should cover [45, 92].

Previous research usually applied electrical stimulation to a large area according to the segment level [10, 27, 39, 40, 49]. Even though some researchers have tried to develop electrode arrays to apply stimulation to a smaller area, the spatial resolution of these electrodes was limited to 1 mm [24, 92]. Because applying single-neuron stimulation to all sensory inputs separately and simultaneously was technically difficult, we attempted to restrict the stimulation area as small as possible to see the effects of spatial resolution on selectivity of hindlimb-related neural circuits.

To apply optogenetics, we used ChR2 transgenic animals. This light-gated ion channel can be activated by light with a wavelength of $460 \pm 50\,nm$ and light power of 1 to 5 mW/mm^2 [64, 95].

Based on a previous behavioral test in optogenetics, a light duration of 50 ms at 10 Hz was sufficient to induce light-dependent behavior [42]. For epidural electrical stimulation, a pulse duration of 0.2 ms at 40–50 Hz was usually used [10, 24, 27, 39, 40]. We considered a temporal resolution of 0.1 ms to control the light sufficient in our system.

Summary of the requirements specification is listed in Table 3.1.

Table 3.1: Requirements specification.

Parameters	Requirements
Area to cover	$\geq 3.5 \times 12\,mm^2$
Spatial resolution	$\leq 1\,mm$
Light wavelength	$460 \pm 50\,nm$
Light power at the target site	$\geq 5\,mW/mm^2$
Modulation frequency	$\geq 50\,Hz$

3.2 Design of the Multichannel Optical Stimulation System

The optical stimulation system was comprised of three parts: a light source that generated light that evokes ChR2 activity, a light-delivery interface that delivered light from the source to the animal spinal cord, and a control system that controlled the power and exposure spot of the light (Figure 3.1).

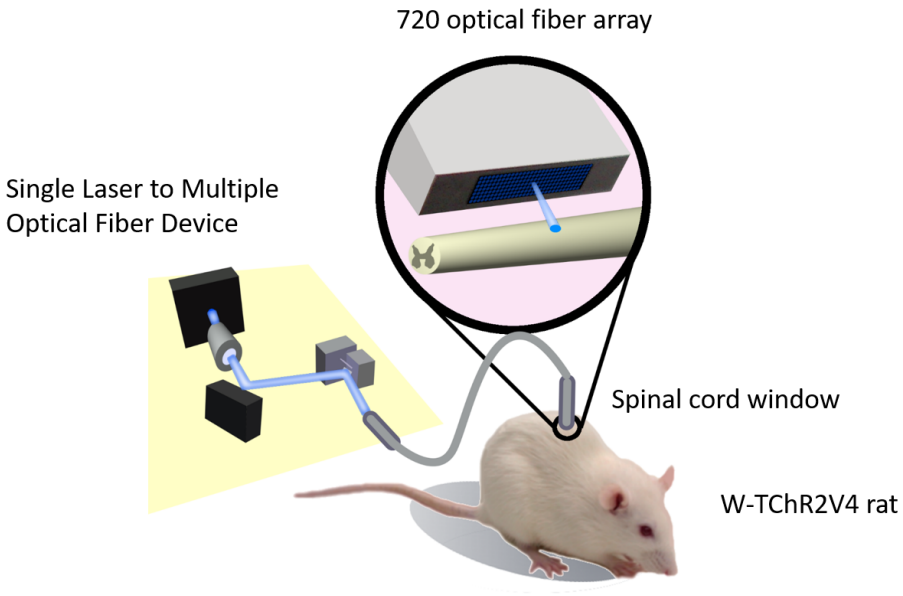


Figure 3.1: System outline [7].

3.2.1 Light Source

A variety of light sources, including lasers, light-emitting diodes (LEDs), and incandescent sources, have been considered in optogenetics research. LEDs, which have been used in several devices, have the advantage of being simple and inexpensive, but the relatively high heat generation, high beam divergence, and broad emission patterns limited their usage in our device [95]. In contrast, a laser diode (LD) can produce a narrow and focused beam, which is suitable to our purpose of targeting more detailed stimulation spots on the spinal cord.

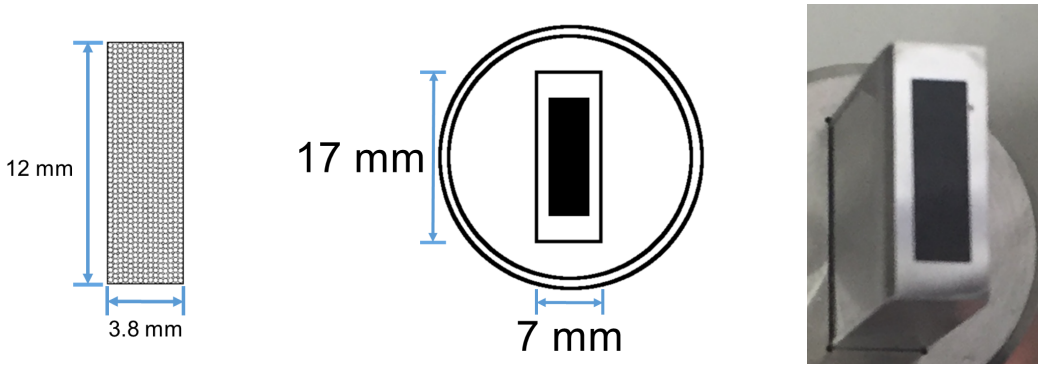
We therefore selected a blue laser diode (PL 450B, OSRAM) as the light source. This LD has an output power of 80 mW, a typical emission wavelength of 450 nm, and a modulation frequency greater than 100 MHz. The 450-nm wavelength is

covered by the absorption spectrum of ChR2. We mounted and drove the LD with an LD/temperature controller set (LTC100-B, Thorlabs) to guarantee a stable light output. The emitted LD light was collimated by an aspherical lens with a focal length of 4.5 mm (C230TMD, Thorlabs).

3.2.2 Light Delivery

Owing to the size of the LD and the related parts to steer the shape of the laser beam, directly mounting the LD on a live animal was inappropriate. We utilized optical fibers with a core diameter of 200 µm and outer diameter of 250 µm, to deliver light from the LD to the animal spinal cord.

To access the different spots of the hindlimb-related neural circuits, multiple optical fibers were arranged into an array. A total of 720 optical fibers were used and arranged in a 15×48 matrix, which resulted in an area of $3.8 \times 12\, mm^2$ (Figure 3.2). These optical fibers were embedded into a rigid head to fix the position and protect them from impact force. Subsequently, a bendable metal tube harnessing the fibers connected the rigid head to the optical source (Figure 3.3(a)). The designed fiber bundle was fabricated by Mitsubishi Cable Industries, Ltd. (Figure 3.3(b)).

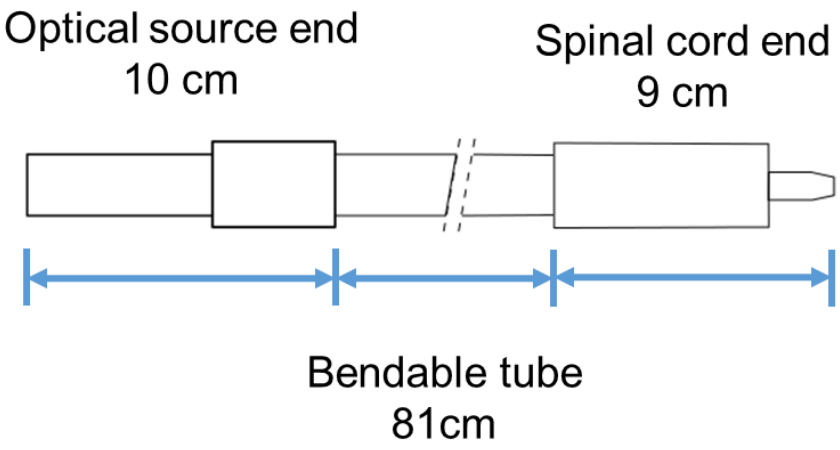


(a) (b) (c)

Figure 3.2: Design of the optical fiber array [6]. (a) The optical fibers were arranged in a 15×48 matrix. (b) Front view of the array. Optical fibers were embedded in the black area. (c) Fabricated optical fiber bundle head

3.2.3 Single-Laser-to-Multiple-Optical-Fiber Device

The collimated beam from the LD (PL 450B, OSRAM) cannot be used directly because the beam is originally elliptical and larger than the optical fiber. To direct the laser beam into the target optical fiber, several optical elements were used. First, an anamorphic prism pair (PS879-A, Thorlabs) was installed to transform



(a)

(b)

Figure 3.3: Design of the optical bundle tube [6]. (a) The optical fiber bundle was designed into a 1 m-long tube. (b) Fabrication of the optical fiber bundle.

the elliptical laser beam into a nearly circular beam. Next, a convex lens was used to converge the beam waist to match the size of the optical fibers (diameter 200 µm).

Instead of preparing 720 LDs to provide light independently to each optical fiber, we utilized only one LD and changed its projection direction to the selected spot. This single-laser-to-multiple-optical-fiber (SLMOF) method can dramatically suppress the cost and reduce the device size. A dual-axis Galvo system (GVS202, Thorlabs), which was composed by rotatable X and Y-axis mirrors, was set to change the direction of light projection. When the light traveled to the Galvo system, it would be reflected 2 times by the X and Y-axis mirrors respectively to our desired direction. These mirrors can respond to 1-kHz signal manipulation. The design diagram of the optical table is shown in Figure 3.4.

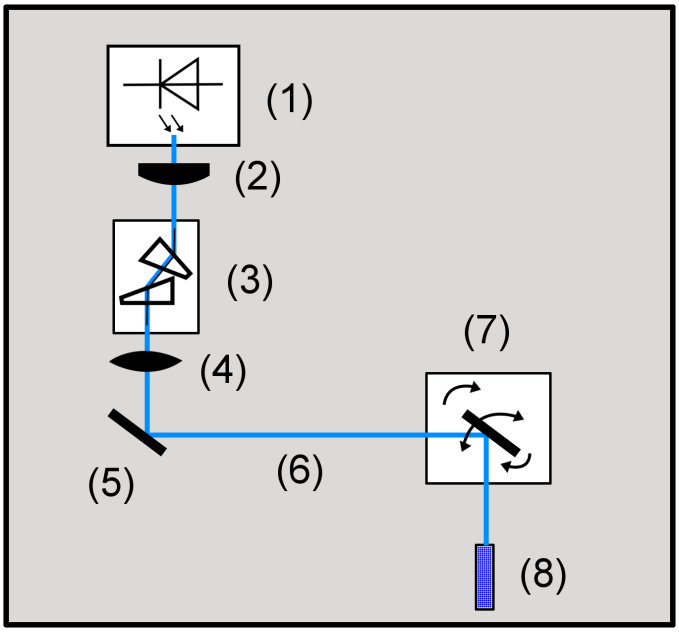


Figure 3.4: Design of the SLMOF device [7]. The device includes the following main elements: (1) LD, (2) collimator lens, (3) anamorphic prism pair, (4) convex lens, (5) right angle mirror, (6) light path, (7) dual-axis Galvo system, and (8) optical fiber bundle (optical source end).

The LD power and the motion of the Galvo system were controlled by a four-channel analog output module (USB-9263, National Instruments). This module can operate with an output voltage between ±10 V in 16-bit resolution, resulting in 305.2 µV for each output step, with an update rate of 100 kS/s.

We set the X and Y-axis mirrors of the Galvo system 135 and 125 mm apart from the optical fiber bundle. Because the distance between the center of fibers was 250 µm (outer diameter of the fiber), the necessary angles for rotating the X and

Y-axis mirrors to project light to adjacent fibers were approximately 0.106 and 0.115 degrees. The mechanical position signal input scale factor was set to 1 V/degree. Therefore, the requirements for the input voltage step were 106 and 115 mV. The control module is thought to have sufficient resolution for fast alterations of laser reflection into the target optical fibers.

Figure 3.5 shows the optical table assembled with all necessary elements. The control software of the system was written in C++ with NI-DAQmx.

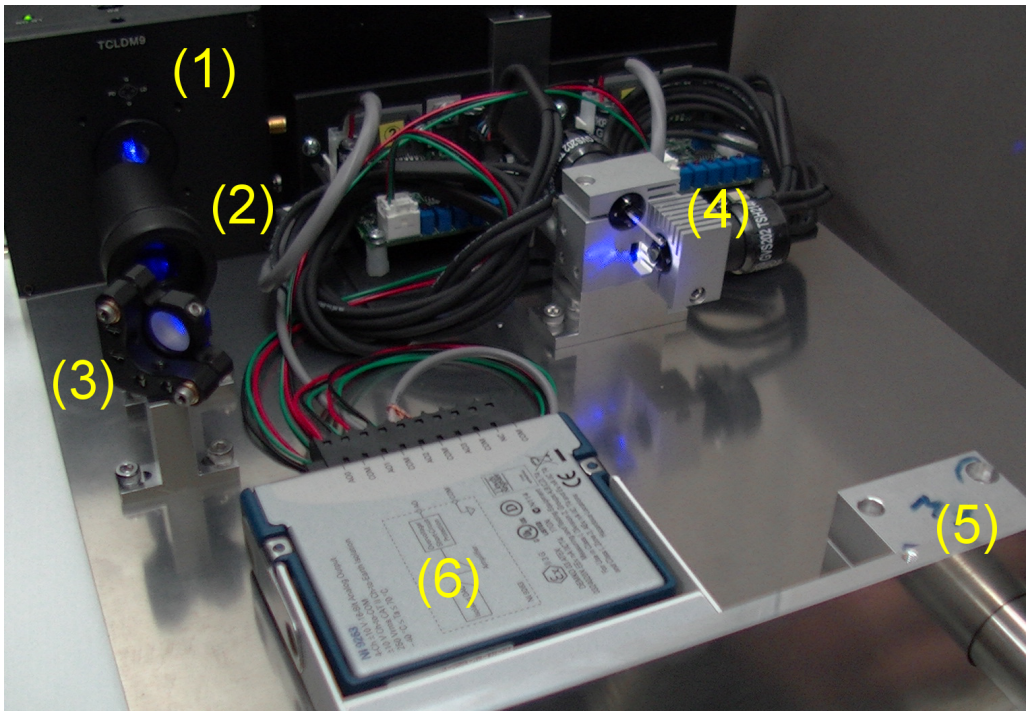


Figure 3.5: The inside view of the SLMOF device with all the optical elements being assembled [7]. The main elements include: (1) LD and collimator lens, (2) anamorphic prism pair and convex lens, (3) right angle mirror, (4) dual-axis Galvo system, (5) optical fiber bundle (optical source end), and (6) NI USB-9263.

3.3 Optical Output Evaluation

After we manufactured the device, we evaluated the output performance of the device and determined the proper control current and projection angle of the laser to each fiber.

3.3.1 Relation Between Control Current and Output Light Power

We first defined the lower-left fiber as location (x,y)=(1,1) and the upper-right fiber as location (15,48). Then, we manually adjusted the laser to project to location (1,1) and measured the light output from the other end of the fiber by using a photodiode laser measurement sensor (PD300-1W, Ophir) and power meter (Nova, Ophir). The resultant value was then divided by the area of the optical fiber to calculate the light power density.

The relation between the light power and control current was linear after the threshold current of 30 mA was exceeded. An input current of 40 mA resulted in a light output of 40.8 mW/mm^2, whereas an input current of 80 mA resulted in an output of 275.4 mW/mm^2 (Figure 3.6). Because previous studies have indicated that approximately 1–5 mW/mm^2 of light power is sufficient to elicit action potentials of ChR2 [95], the output of our device is considered sufficient.

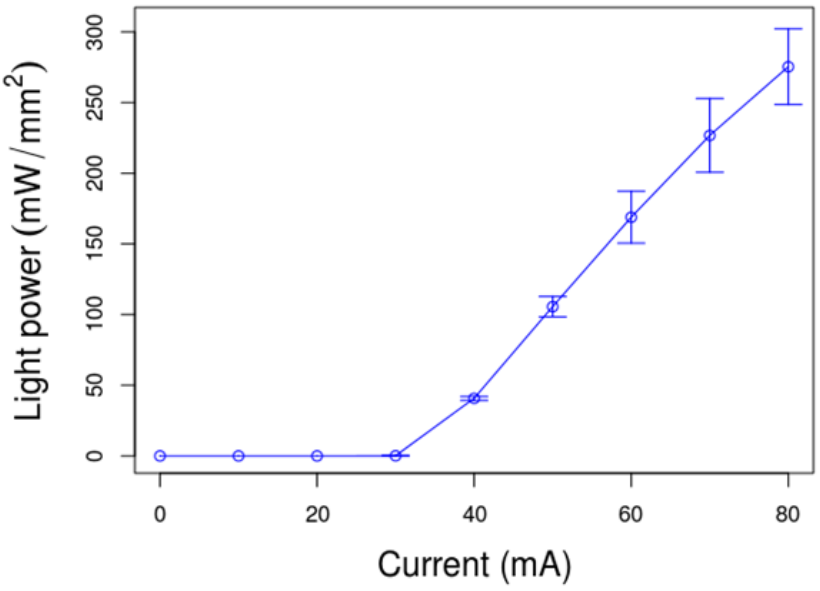


Figure 3.6: Light power density under different control currents [7]. ($n = 4$; $mean \pm S.D.$)

3.3.2 Calibration of Light Power Output in Multiple Fibers

After we evaluated the relation between the control current and the output light power, we wanted to confirm the output light power of each fiber.

We first determined the incident angles of the laser to fibers located at (1,1), (1,48), (15,1), and (15,48) manually. It was assumed that the proper incident angle of the laser to fiber would be that projected to the center of each fiber. And furthermore, the incident angles to project the light to adjacent fibers were approximately equal because the distance from the Galvo system to the fiber bundle is relatively large compared to the size of the end section of the bundle. As a result, the incident angles to the others could be calculated by dividing the distance between these endpoints with the number of fibers between them.

The light output from the other end of each fiber controlling by the method mentioned above was measured under a control current of 80 mA. However, we found an inconsistent output pattern (Figure 3.7).

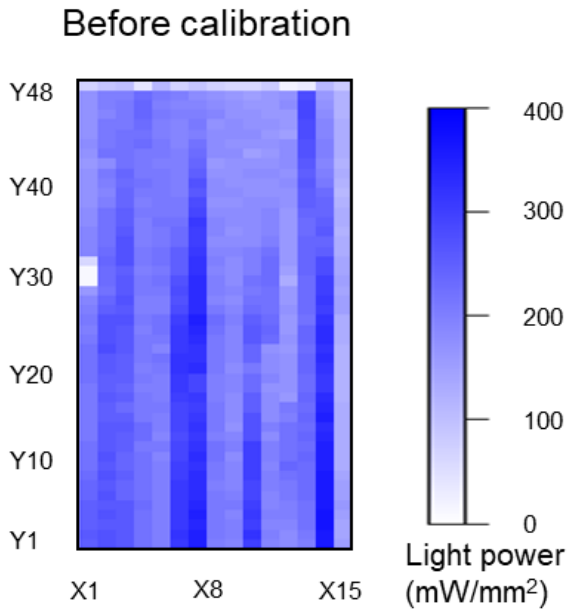


Figure 3.7: Optical power output under the same control current of 80 mA before calibration [6].

This inconsistent output pattern might be caused by inappropriate incident angles or differences in the construction of each fiber. To amend the problem, a calibration process that automatically determined the best incident angles and adjusted the appropriate control current was necessary. This process is similar to the automatic fiberoptic alignment problem, which usually has been solved by the gradient search algorithm [23].

We adopted a hill-climbing-based algorithm to calibrate the system. The process began by calculating the theoretical laser incident angle to the center of each fiber

as the starting point and subsequently hill-climbing to search for a better incident angle for higher light power output. After we found the optimal incident angle to produce maximum output from a fiber, we changed the control current of laser and measured the corresponding output to calculate the the linear correlation between control current and light power output of each channel (Figure 3.8).

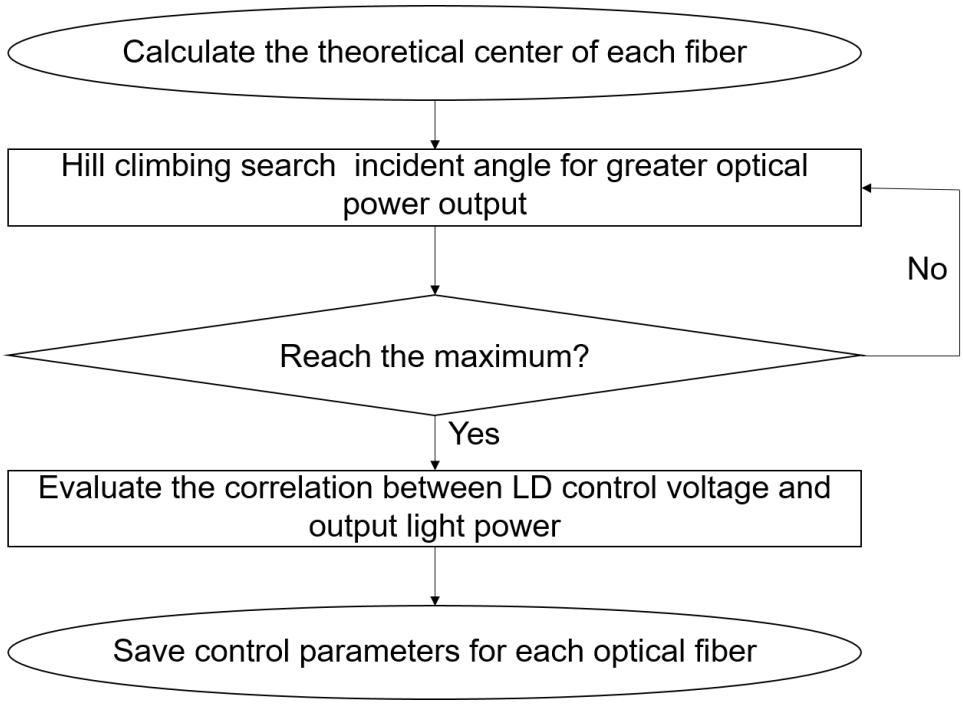


Figure 3.8: Light power output calibration process.

We used the control parameters from calibration to test the designated light power output for each fiber. Under max control current (80 mA), all channels could produce a light power output that exceeded the necessary value to evoke ChR2 activity, and the consistency between them also showed a dramatic improvement (Figure 3.9).

3.4 Summary and Discussion

Our objective was to modulate spinalized lower-body motion by stimulating the related epidural region. The ideal method was to separate all single nerves and stimulate the target nerve so that the other nerves are not affected. However, this is extremely difficult with the current technique of neuromodulation because there were thousands of neurons, which were related to the sensory input from the lower body, gathered in a small area of the spinal cord segments. Consequently, we selected the second-best way of increasing the spatial resolution to the maximum.

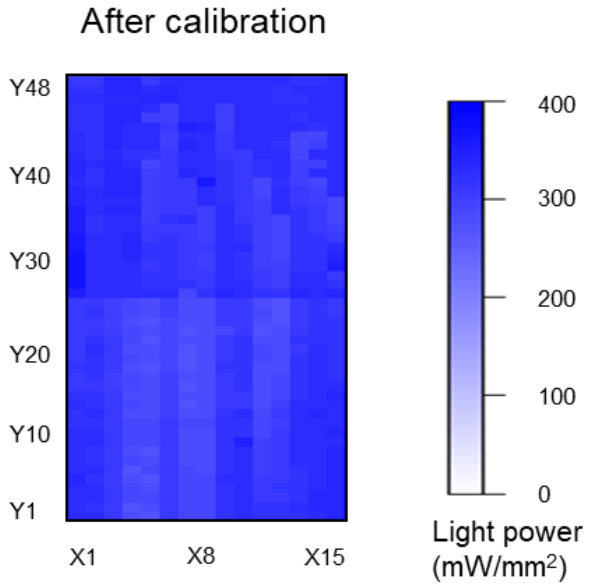


Figure 3.9: Using parameters that produce the 300 mW/mm^2 optical fiber output from each fiber to evaluate the performance after calibration [6].

We proposed the use of multichannel optogenetic stimulation to elevate the spatial resolution. The proposed device contained 720 optical fibers to cover the locomotion-related spinal cord area in rats. Compared to that of a previous research, which used a maximum of 27 electrode arrays [24, 34, 92], our device had 26.7 times higher spatial resolution.

To produce light output with stable high power and low divergence, we chose the LD as the light source. The drawback of this selection was the large size and high cost compared to other light sources, such as LEDs. To overcome this, we designed an SMLOF method to drive the system. By carefully steering the laser beam in the device and automatically calibrating the output with a machine-learning-based process, we could generate high spatiotemporal light output for use in optogenetic spinal cord stimulation.

The capability of our system is summarized in Table 3.1.

Table 3.2: Specifications of the SLMOF system.

Parameters	Requirements	Specifications
Area to cover	$\geq 3.5 \times 12\, mm^2$	$3.8 \times 12\, mm^2$
Spatial resolution	$\leq 1\, mm$	200 µm (With 720 points.)
Light wavelength	$460 \pm 50\, nm$	450 nm
Light power	$\geq 5\, mW/mm^2$	$\geq 300\, mW/mm^2$
LD modulation frequency	$\geq 50\, Hz$	$\geq 100\, MHz$
Fiber length	Long enough for different experimental conditions	1 m flexible fiber bundle

Chapter 4

Light Propagation in Spinal Cord Tissue

The content of this chapter is concealed because it is scheduled to be published in the form of a journal in 3 years.

この章の内容は 3 年以内に学術雑誌等において出版予定であるため割愛いたします.

Chapter 5

Animal Model for Optogenetic Spinal Cord Stimulation

The content of this chapter is concealed because it is scheduled to be published in the form of a journal in 3 years.

この章の内容は 3 年以内に学術雑誌等において出版予定であるため割愛いたします.

Chapter 6

Examining the Muscle Reaction Induced by Optogenetic Spinal Cord Stimulation

The content of this chapter is concealed because it is scheduled to be published in the form of a journal in 3 years.

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Chapter 7

Analyzing the Resolution of Multichannel Optogenetic Spinal Cord Stimulation

The content of this chapter is concealed because it is scheduled to be published in the form of a journal in 3 years.

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Chapter 8

Discussions

The content of this chapter is concealed because it is scheduled to be published in the form of a journal in 3 years.

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Chapter 9

Conclusions

The content of this chapter is concealed because it is scheduled to be published in the form of a journal in 3 years.

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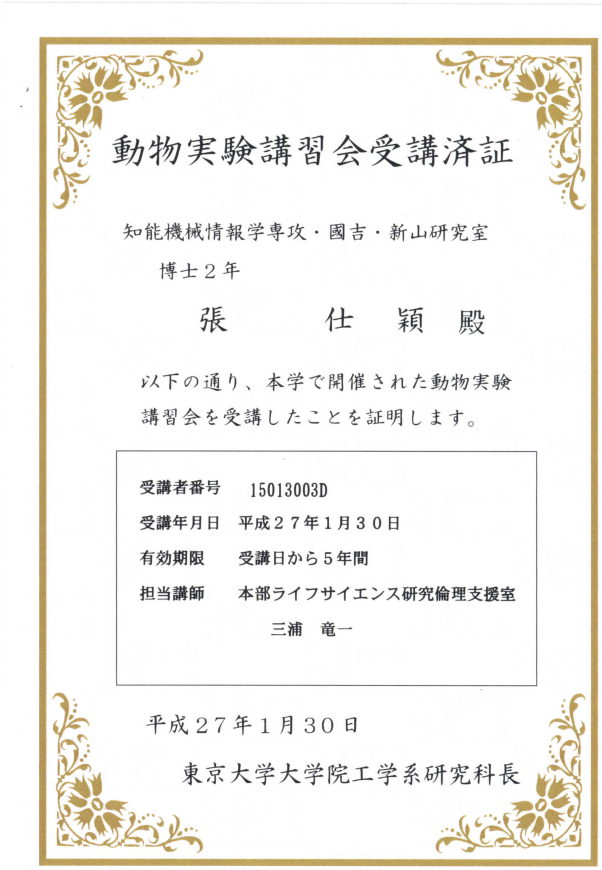
Appendix A: Education and Training for Experiments

Table App.A.1: Completed education and training for experiments.

Course	Note
安全衛生の基本講習	
レーザー講習会	
動物実験講習会	Figure App.A.1
遺伝子組換え実験講習会	
化学物質講習会	
環境安全講習会	Figure App.A.2



動物実験講習会受講済証

知能機械情報学専攻・國吉・新山研究室

博士２年

張　　仕　穎　殿

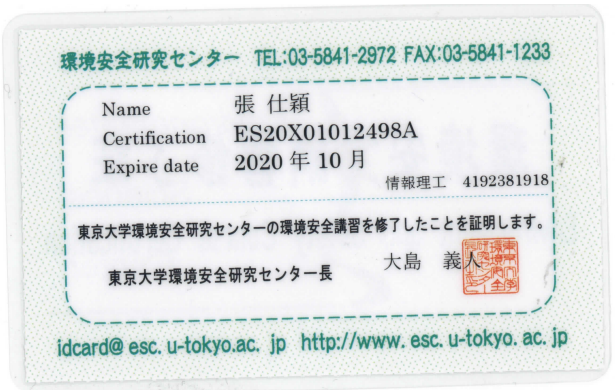
以下の通り、本学で開催された動物実験講習会を受講したことを証明します。

受講者番号	15013003D
受講年月日	平成２７年１月３０日
有効期限	受講日から５年間
担当講師	本部ライフサイエンス研究倫理支援室 三浦　竜一

平成２７年１月３０日

東京大学大学院工学系研究科長

Figure App.A.1: Completion certificate of animal experiment education.



環境安全研究センター　TEL:03-5841-2972 FAX:03-5841-1233

Name　張 仕穎

Certification　ES20X01012498A

Expire date　2020 年 10 月

情報理工　4192381918

東京大学環境安全研究センターの環境安全講習を修了したことを証明します。

東京大学環境安全研究センター長　大島　義人

idcard@ esc. u-tokyo.ac. jp　http://www. esc. u-tokyo. ac. jp

Figure App.A.2: Completion certificate of environment and safety course.

Appendix B: Application and Approval of Animal Experiment

Animal experiments in this thesis were conducted in accordance with the University of Tokyo's guidelines regarding animal research (東京大学動物実験実施マニュアル, `http://anzen2.t.u-tokyo.ac.jp/anzen2/experiment/lifescience/animal_experiment/index.html`). The experimental protocol was approved by the Animal Experimentation Committee of The University of Tokyo (Table App.B.1).

Table App.B.1: List of approved applications regarding animal experiment.

Applications	Permit Number
動物実験計画書	KA14-4, KA19-1
動物実験従事者変更計画書	KA14-4-1
動物実験変更計画書	KA14-4-2, KA14-4-3
第二種使用等拡散防止措置 機関承認実験	KB17-8, KB19-1

Appendix C: Detection Rate of the EMG Response Induced by Optical Stimulation.

The content of this chapter is concealed because it is scheduled to be published in the form of a journal in 3 years.

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Appendix D: EMG Intensity of the Muscle Response Induced by Optical Stimulation.

The content of this chapter is concealed because it is scheduled to be published in the form of a journal in 3 years.

この章の内容は 3 年以内に学術雑誌等において出版予定であるため割愛いたします.

Appendix E: Abbreviations Used in the Thesis

Table App.E.1: List of abbreviations.

Abbreviations	Full name
ASIA	American Spinal Injury Association
BF	Biceps femoris
BMI	Brain machine interface
C	Cervical
ChR2	Channelrhodopsin-2
CNS	Central nervous system
CPG	Central pattern generators
DRG	Dorsal root ganglion
EES	Epidural electrical stimulation
EMG	Electromyogram
ER	Early response
g	Anisotropy

Table App.E.2: List of abbreviations.

Abbreviations	Full name
ISNCSCI	International Standards for Neurological Classification of Spinal Cord Injury
L	Lumbar
LD	Laser diode
LED	Light-emitting diodes
LR	Late response
MG	Gastrocnemius medialis
MR	Middle response
n	Refractive index
PDMS	Polydimethylsiloxane
PNS	Peripheral nervous system
S	Sacral
SCI	Spinal cord injury
SCS	Spinal cord stimulation
SCW	Spinal cord window
SLMOF	Single Laser to Multiple Optical Fiber
T	Thoracic
TA	Tibialis anterior
VL	Vastus lateralis
μ_a	Absorption coefficient
μ_s	Scattering coefficient

Appendix F: Publication List

Refereed Conference

1. Shih-Yin Chang, Satoshi Nishikawa, Masaki Sekino, Hiroshi Onodera, and Yasuo Kuniyoshi: "A Simulation Study of Light Propagation in the Spinal Cord for Optogenetic Surface Stimulation", The 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2019, pp. 6872-6875.

2. Shih-Yin Chang, Kazunori Naganuma, Hoshinori Kanazawa, Masaki Sekino, Hiroshi Onodera, and Yasuo Kuniyoshi: "Applying Multichannel Optogenetic System for Epidural Spinal Cord Stimulation in Rats", The 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2018, pp. 1440-1443.

3. Shih-Yin Chang, Kazunori Naganuma, Hoshinori Kanazawa, Kenta Takashima, Kuniaki Konishi, Takao Someya, Masaki Sekino, Yasuo Kuniyoshi, and Hiroshi Onodera: "Single Laser to Multiple Optical Fiber Device for Optogenetics-based Epidural Spinal Cord Stimulation", The 8th International IEEE EMBS Conference on Neural Engineering (NER), 2017, pp. 207-210.

4. Shih-Yin Chang, Kenta Takashima, Satoshi Nishikawa, Ryuma Niiyama, Takao Someya, Hiroshi Onodera, and Yasuo Kuniyoshi: "Design of Small-Size Pouch Motors for Rat Gait Rehabilitation Device", The 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2015, pp. 4578-4581.

Non-refereed Conference

1. 張仕穎、西川鋭、新山龍馬、國吉康夫: "小型パウチモータの性能向上のための分割設計", 第13回身体性認知科学と実世界応用に関する若手研究会 (ECSRA), 2015.

2. 張仕穎, 西川鋭, 國吉康夫: ”片足障害による歩行パターンの変化についての運動解析”, 第 12 回身体性認知科学と実世界応用に関する若手研究会 (ECSRA), 2014.

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