POLYCRYSTALLINE DIAMOND BASED NEURAL INTERFACE FOR OPTOGENETICS AND NEUROTTRANSMITTER DETECTION

By

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ABSTRACT

POLYCRYSTALLINE DIAMOND BASED NEURAL INTERFACE FOR OPTOGENETICS AND NEUROTRANSMITTER DETECTION

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Neural interface forms a communication bridge between a human brain and external circuitries, which enables promising bioelectronics medicines for diseases treatments, such as inflammatory bowel disease, Alzheimer's disease, and restore sensorimotor function lost due to traumatic brain, spinal cord injury, and amputations. Neurons in the central nervous systems communicate with each other electrically along the axon from soma to dendrite and chemically between neuron to neuron in the synapses through release and uptake of neurotransmitters. In particular, dopamine (DA) is one of the most important neurotransmitters, which associates with many aspects of the neurophysiological processing, such as stress, memory, and addiction. External stimulation is desired to study the dynamics of DA release and uptake and its correlation to the animal behavioral changes. Previously, electrical stimulation was used as a neuromodulation technique for such purpose, which can cause a significant amount of nondopaminergic system activation and result in consequential neurological activities or dynamics not related to DA release[1]. Recent advances in optogenetics provide a unique neuromodulation technique, allowing optical control of genetically targeted specific neurons that express light-sensitive opsin proteins with sub-millisecond temporal precision. Utilizing the cell-type specificity of Optogenetics, researchers can have a more controlled manipulation of the dopaminergic system and have an unbiased study on DA related neurological diseases.

The current engineering tools for Optogenetics use laser and micro light emitting diodes (μLEDs) as the light sources, where μLEDs show great promises with respect to device
miniaturization, simplicity, low power and low cost of system implementation. However, using μLEDs as a light source can cause potential thermally-induced tissue damage due to μLED Joule heating. To address the localized Joule heating issue, a μLED based optrode was developed in this thesis using polycrystalline diamond as a heat spreader due to its very high thermal conductivity. Compared with an SU8 probe with the same dimensions, a diamond probe can reduce the maximum temperature variations by ~90% at 3.6V 100ms duration pulses. The functionality of the probe was tested in vivo, where light-evoked action potentials were successfully detected.

Besides the very high thermal conductivity, diamond has unique features for neurotransmitter sensing, such as a larger potential window, low background current and resistance to surface fouling. In addition, diamond is a biocompatible and chemically inert material, which enables long-term device implantation. Therefore, above mentioned properties make diamond a promising candidate for Optogenetics and neurotransmitter detection. However, diamond is a rigid material and the micromotion-induced strain has been hypothesized to be the main cause of harmful immune responses and even irreversible tissue damage. Due to the process temperature intolerant, diamond cannot synthesis onto polymer substrates directly. To address this issue, a wafer-level substrate transfer process is first time proposed to transfer all diamond macro/micro patterns from a diamond growth substrate, silicon, onto a flexible Parylene substrate. The electrochemical properties of the transferred diamond-polymer electrodes were evaluated (i) using an outer sphere redox couple to study the electron transfer process and (ii) quantitative and qualitative studies of a neurotransmitter redox dopamine/dopamine-o-quinone. A linear response of the BDD sensor to dopamine concentrations of 0.5 µM to 100 µM was observed (R² = 0.999) with a sensitivity of 0.21 µA/cm²·µM.
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The completion of my Ph.D. program at Michigan State University is just a start of another new journey of my way. I would like to quote what Andy said in the movie of Shawshank Redemption at the beginning of this new journey: “Hope is a good thing, maybe the best of things, and no good thing ever dies.”
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Chapter 1. Introduction

1.1 Background

The complex brain networks comprise billions of interconnected neurons with diverse types, shapes, sizes, and activity patterns. Targeted access to specific neural populations with high spatiotemporal resolution enables the study of neural circuits and cellular conditions, for both fundamental understandings of brain functions and development of therapeutic strategies for many brain injuries and disorders. While well-established microelectrophysiological methods have been successfully used to record neural activity at single-cell resolution[2], neuromodulation with electrical modality, which initiates neural functional response by injecting a biphasic current to depolarize the membranes of nerve cells[3], suffers from indiscriminate stimulation of cell components (somas, dendrites, and axons) as well as poor spatial resolution due to unpredictable current pathways[4]. Recent advances in optogenetics provide a unique neuromodulation technique, allowing optical control of genetically targeted specific neurons that express light-sensitive opsin proteins, such as light-sensitive ion channels, Channelrhodopsin-2 (ChR2)[5], optically activated chloride pumps, Halorhodopsin (NpHR)[6] and proton pumps, Archaerhodopsin (Arch)[7]. The cell-type specificity of Optogenetics is achieved by selecting appropriate promoters, for example, CamKIIα for targeting excitatory neurons, glial fibrillary acidic protein for targeting astroglia, and ppHcrt promoter for targeting hypocretin neurons in rodents[8].

At the same time, neurons in the central nervous systems communicate with each other electrically along the axon from soma to dendrite and chemically between neuron to neuron in the synapses through release and uptake of neurotransmitters. In particular, dopamine (DA) is one of the most important neurotransmitters. Dopamine (DA) is contracted from 3,4-
dihydroxyphenethylamine associated with many aspects of the neurophysiological processing, such as stress[9], memory[10], and addiction[11]. There are three major dopaminergic pathways in the brain: Mesolimbic pathway from ventral tegmental area (VTA) to the nucleus accumbens (NAc), Nigrostriatal pathway from substantia nigra (SN) to the striatum, Mesocortical pathway from VTA to the prefrontal cortex[12]. Abnormal activities of DA storage, release and reuptake are the main cause of several neural disorders in the central nervous system, such as Parkinson’s diseases and schizophrenia [1],[11]. Besides, dysregulated DA is found to be an important factor affecting cardiovascular and renal systems. For example, a DA dose between 32 to 64 µg/kg caused an increase in heart contractile force, heart rate, and arterial pressure of an anesthetized dog[13]. Low-dose DA has been commonly used to increase renal blood flow and reduce the risk of renal failure[14]. Hence, real-time monitoring of dynamic changes in DA concentration is very important for understanding the functionality of the brain and other organs. Because of the low concentration and dynamically changing levels of DA in the brain, in-situ detection without sample treatment is desired, which requires implanted sensors with a wide working potential window, resistance to molecular adsorption and corrosion, biocompatibility, mechanical flexibility, high sensitivity and selectivity of the target analyte. Of well-established chemical sensing approaches, electrochemical sensors show unique advantages of low cost, fast dynamic response (up to the millisecond range), miniaturized geometry, and high spatial resolution.

External stimulation is desired to study the dynamics of DA release and uptake and its correlation to the animal behavioral changes. Previously, electrical stimulation was used as a neuromodulation technique for such purpose, which can cause a significant amount of nondopaminergic system activation and result in consequential neurological activities or dynamics not related to DA release[1]. Utilizing the cell-type specificity of Optogenetics,
researchers can have a more controlled manipulation of the dopaminergic system and have an unbiased study on DA related neurological diseases. Specifically, Threlfell et al. [15], have demonstrated that light-induced activation of cholinergic interneurons can trigger dopamine release in mouse striatum through the activation of nicotinic receptors. Bass et al. [16] have studied optically evoked DA release in rat substantia nigra with different quantities and light pulse width, where dopamine release is found to be more sensitive to the changes of the optical pulse width. Melchior et al. [17] have compared electrical and optical stimulation on dopamine terminals in the nucleus accumbens. Lu et al.[1] have studied different optical light stimulation parameters on direct manipulation of DA release and dynamic in the nucleus accumbens, such as light intensity, pulse width, and the shape of stimulation waveforms.

1.2 Current challenges on hardware development

In order to fully realize the remarkable potential of studying DA dynamics using Optogenetics, engineering tools are in demand to achieve simultaneous light delivery, electrophysiological and neurochemical recording. Despite the significant development of a wide variety of Optogenetics and Neurotransmitter detection tools, several challenges still remain such as localized heating due to LED activation, material compatibility and safety, and electrochemical performance of the electrodes. The following sections will discuss these challenges in the current approaches and envision possible solutions to the identified problems.

1.2.1 Thermal challenges of putting µLED near tissue

Recently, advanced microfabrication techniques have been investigated to construct and miniaturize optical neural implants capable of multi-site, localized light stimulation of
three-dimensional (3D) brain networks with fine spatial resolution. These devices can be categorized into two major groups based on different light sources: laser, including laser diodes and diode-pumped solid-state (DPSS) laser diodes, and LEDs, including bulk LEDs and microscale LEDs (µLEDs). Although lasers and laser diodes provide several benefits, including high light intensity, low beam divergence, and narrow spectral bandwidth, laser-based optical systems have the following drawbacks such as high power consumption with typical several tens of mW per channel, difficulty for integrating with wireless telemetry and restriction of natural behavior of the subjects by tethered optical fibers and commutation systems[18]. Compared to laser and laser diodes, LEDs provide unique advantages, including low power consumption, illumination stability, and fast light-switching ability[19]. More importantly, electronically driven LEDs are particularly suitable for integration with wireless telemetries to enable fully implantable systems for applications in freely behaving animals[20]. However, they are not without significant concerns. These include potential thermally-induced tissue damage due to µLED heat deposition in the brain, particularly for high-density neural implants where microelectronics are in direct contact with large-area brain tissues. To prevent tissue damage and consequent behavioral and physiological changes, the temperature perturbation induced by optical neural implants should be less than 1 °C [21][22]. Therefore, there are several important considerations that should be taken into account when designing LED-based optical devices. First, device layout and µLED array configuration can be optimized to minimize electrical heat generated from µLEDs. Second, the proper selection of substrate materials can potentially reduce localized heating effects by dissipating the LED heat into surrounding brain tissue. The high thermal capacity of brain tissue can counteract the temperature variation. Third, optical stimulation
parameters should be optimized to enable effective opsin activation, while preventing the overheating of brain tissue.

In order to reduce electrical heat generated during the operation of μLEDs, the thermal performance of μLEDs has been explored analytically and experimentally[23][24][25][26]. LEDs with different dimensions were fabricated[25] on a poly(ethyleneterephthalate) (PET) substrate. The thermal performance was quantified by measuring the maximum temperature change upon activating the LEDs under different conditions using a thermal imager. The following findings are derived from these studies. First, increasing the LED size can lead to an increase in the maximum temperature change and a decrease in the overall energy efficiency. Second, when designed in an array configuration, increasing the separation between μLEDs can effectively decrease the maximum temperature change. Finally, decreasing the pulse duty cycle can also reduce the maximum temperature rise.

In addition, analytical and finite element method (FEM) simulations[23] have been conducted to predict the thermal behavior of μLEDs and μLED arrays in tissues. Both approaches imply that the maximum temperature change in tissues can be reduced by lowering the peak power and decreasing the duty cycle and period of LED activation. For a μLED array, a larger $r_d/\sqrt{A}$ will result in a smaller temperature change, where $r_d$ is the distance between the centers of two adjacent μLEDs and $A$ is the total surface area of the μLED. To further reduce the temperature variation during optical stimulation, especially the localized hot spots, a substrate material with high thermal conductivity should be carefully selected such as polycrystalline diamond, which has a thermal conductivity (up to 1800 W/(m·K)) [27].

1.2.2 Material long-term compatibility and safety

One of the major challenges of fiber- or waveguide-coupled optical systems is to obtain high
optical coupling efficiency from the fiber (or waveguide) to the stimulation site. Microfabricated fibers and waveguides are normally made of polymers, such as SU-8, or dielectric materials, such as oxynitride. While polymers provide excellent mechanical flexibility and fabrication simplicity, the absorption of water could negatively affect the long-term optical properties of the polymer-based devices. Deterioration of mechanical properties of polymer waveguides is also observed during aging of the devices in buffered saline solution (PBS). Moreover, commonly used photosensitive polymers, such as SU-8, have a high absorption loss near 473 nm[28][29], which significantly reduces their light-guiding quality. Finally, the biocompatibility of SU-8 has not been fully evaluated in chronic studies. Dielectric materials are considered to be more appropriate than polymers because of their biocompatibility, low water permeation and absorption rates, and optical clarity over a broad spectral region. However, thick dielectric waveguides are difficult to construct due to stress and extended plasma-etching time. As a result, the coupling efficiency between thin dielectric waveguides and multi-mode fiber optics can be significantly affected by the large coupling loss at the fiber-waveguide junction. Further modification and optimization of fabrication techniques are necessary to improve the coupling efficiency. Furthermore, silicon-based dielectric films have shown increased dissolution in water at elevated temperatures and may require additional encapsulating barriers for chronic applications[30].

Another major challenge of chronic neural implants is the mechanical property mismatch between rigid implanted devices and soft brain tissue, which increases the possibility of negative neural response, glial scar formation, inflammation, and mechanically induced trauma[31][32][33]. While the mechanical rigidity can be alleviated by the use of polymer substrates, the surgical insertion of such flexible devices into deep brain regions will be challenging. To address this issue, a temporary coating that can stiffen the probe during the
insertion and be dissolved by body fluids afterward has been adopted to facilitate the implantation of the flexible optical neural implants. Among different biodegradable polymers, silk fibroin, a biopolymer obtained from cocoons, has been widely used in bio-integrated electronics[34]. Silk fibroin can be dissolved by most aqueous solutions with a programmable rate of dissolution controlled by the ratio of solvent and silk concentrations. Tae-il Kim et al. [26] successfully demonstrated the use of silk to temporarily bond a flexible µLED probe to a thick and rigid epoxy carrier during probe insertion. The silk fully dissolved in an artificial cerebrospinal fluid (ACSF) solution 15 min after the insertion was made. Another dissolvable adhesive, polyethylene glycol (PEG), was used by Falk Barz et al. [35]. In this study, the PEG with a molecular weight (MW) of 1500 g/mol was quickly dissolved in electrically conducting agar-based gel in 1 min. As the melting point of PEG with different MW can range from 4–8 °C (MW=400) to 55–62 °C (MW=8000) [36], a careful selection should be conducted to match the temperature range of the target implantation sites.

Furthermore, as implantable devices get miniaturized, the amount of water needed to increase the humidity of the encapsulated environment decreases accordingly, which takes a shorter time for implanted materials to reach corrosive levels [37]. Therefore, encapsulating materials and techniques should be carefully considered in order to achieve long-term stability of implantable devices. Although traditional processes such as glass-to-metal seal, ceramic-to-metal seal, and fusion welding can provide real hermetic sealing for implantable devices, the high processing temperature may not be compatible with polymer-based implantable devices. Recently, polymer encapsulations, such as Parylene, polyimide, silicone and epoxy, have been widely used as a barrier coating for electronics. Although the biostability of these materials is questionable because polymers tend to degrade due to hydrolytic, oxidative, and enzymatic mechanisms[38], recent
studies have shown that Parylene encapsulation of CMOS circuitry can survive at 55 °C for five months. Besides, metal coated Parylene barriers may further reduce the permeability of moisture and can remain intact in vivo for over 10 years[39]. Atomic-layer-deposited alumina-Parylene bilayer encapsulation has also been studied, where a Utah electrode array (UEA) with an ASIC chip survived for 228 days of soaking testing at 37 °C[40].

Finally, biocompatibility has always been an important criterion of all the implantable devices to prevent glial formation and other foreign body reactions that present significant risks for devices and host tissue. Particularly for Optogenetics applications, glial encapsulation can increase the backscattering and attenuate light delivered to host tissue[41]. Common strategies for minimizing foreign body responses include careful selection of biomaterial coatings, surface modification, and optimization of device design to reduce the size and mechanical mismatch. Considerable work on biomaterials and biocompatibility issues for neural implants has been compiled in [42],[43],[44]

1.2.3 Electrochemical performance of the electrodes

For effective recording and sensing both electrophysiological (electrical) and neurophysiological (chemical) signals, an ideal electrode should have the following important features[45],[46],[47], [48], [49]: Enough potential windows for sensing the target analyte, smaller double-layer capacitance and high sensitivity to achieve higher signal-to-background-noise ratio, resistance to surface biofouling and miniaturized size for large-scale sensing with high resolution. Many efforts have been put onto microfabrication of miniaturized neural probes for electrophysiological recording at single-cell resolution in vivo[50]. However, there are still limitations on device development of extracellular concentrations of neurotransmitters in vivo[51]. Traditional polymer-based Micro-Electro-Mechanical-Systems (MEMS) devices are often based on metal electrodes[52]. However,
metal electrode requires surface modification or pre-treatment methods to increase the sensing surface and facilitate electron transfer process[53]. For example, Aneliya et al. [54] was reported a platinum sensor modified with conducting polymer poly-(3,4-ethylenedioxythiophene, PEDOT) and inorganic Cu crystals of appropriate size to achieve selective detection of the neurotransmitter dopamine in the presence of ascorbic acid. Besides, the commonly used electrode materials, such as platinum, gold, iridium, iridium oxide, cannot survive from long-term implantation. They often failed due to corrosion, astrogliosis and fibrotic encapsulation[55], [56], [57]. Conductive polymers such as PEDOT are seen to modify the metal surface for better biocompatibility but the stability of such method is still unknown.

1.3 Solutions to the challenges and objective of this work

Carbon materials have been widely used in many biosensing applications[58], especially sp³ carbon, i.e. diamond. Diamond is a unique material with complete sp³ hybridization carbon, which results in an extensive tetrahedral bonding between carbons throughout the lattice and leads to many exceptional properties[59]. Diamond has very high thermal conductivity (up to 1800 W/m·K), which is much higher than most noble metals and biocompatible polymers. A comparison of thermal conductivity of the different material is listed in Table 1-1. Besides, diamond is a biocompatible material without any cytotoxic or hemolytic [60],[61] and is resistant to corrosion and surface adsorption and deactivation[62],[63], which enables device long-term reliability and stability. In addition, diamond can be doped with a dopant (boron for p-type and phosphate for n-type) to conduct electricity. For example, the electrical resistivity of boron doped polycrystalline diamond (BDD) can reach 1.69×10⁻³Ω·cm with a doping level of 6000ppm[64]. More importantly,
BDD has relative smaller double layer capacitance and wider potential window, which gives low background current and a wider range of potentials for electrical neural recording and chemical sensing. Therefore, all of the properties that diamond featured above have been proved to be favorable to implanted neural interfaces for Optogenetics.

Table 1-1 Thermal conductivity of noble metal and polymers [65], [66], [67], [68], [69], [70] [71], [72]

<table>
<thead>
<tr>
<th>Material</th>
<th>Thermal conductivity</th>
<th>Material</th>
<th>Thermal conductivity</th>
</tr>
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<tbody>
<tr>
<td>Silicon[65]</td>
<td>149 (W/m·K)</td>
<td>Copper[66]</td>
<td>385 (W/m·K)</td>
</tr>
<tr>
<td>Gold[67]</td>
<td>314 (W/m·K)</td>
<td>Parylene[68]</td>
<td>0.084 (W/m·K)</td>
</tr>
<tr>
<td>Iridium[69]</td>
<td>147 (W/m·K)</td>
<td>SU8[70]</td>
<td>0.3 (W/m·K)</td>
</tr>
<tr>
<td>Platinum[71]</td>
<td>71.6 (W/m·K)</td>
<td>Polyimide[72]</td>
<td>0.12 (W/m·K)</td>
</tr>
</tbody>
</table>

Despite its many advantages, BDD is a rigid material with Young’s modulus of ~1000 GPa [73], which is several orders of magnitude higher than that of the brain tissues (~103 to 105 Pa [74]). The micromotion-induced strain between rigid implants and surrounding soft tissues has been hypothesized to cause a harmful immune response and even irreversible tissue damage[75]. Recently, mechanically flexible, polymer-based neural implants have shown promises as the next generation of implanted devices[43],[76],[77],[78]. For those devices, electrodes and interconnecting traces made of noble metals were constructed on soft polymeric substrates with low Young’s moduli, such as polydimethylsiloxane (PDMS) (360-870 KPa [79]), polyimide (2.5 GPa [72]), SU-8 (SU-8 2000, 2.0 GPa [80]) and Parylene (2.8 GPa [81]). Consequently, the overall effective Young’s modulus can be significantly reduced to minimize the mechanical mismatches between rigid metal and soft tissues. Unfortunately, unlike noble metals, BDD cannot be fabricated directly on a polymer substrate due to its high synthesis temperature (500 – 900 °C [82]) exceeding
the glass transition temperatures of polymers. To address this issue, a wafer transfer process is required to transfer diamond from BDD growth substrates, such as silicon, onto flexible polymer substrates.

The ultimate goal of this thesis work is to fabricate an integrated diamond neural interfacing system using the diamond on a flexible Parylene substrate, which combines three modules- a μLED for optical stimulation with diamond traces as a heat spreader, neurophysiological and neurochemical sensing capability with diamond electrodes. A concept diagram is shown in Figure 1-1, where only one probe is shown in the figure to emphasize the functionality of each module. The work presented in this thesis will be the design and implement of each module, which includes a hybrid neural interface optrode with a polycrystalline diamond (PCD) heat spreader and a novel large-scale wafer transfer process for fabrication of diamond-polymer chemical sensors.

Figure 1-1 Concept diagram of a diamond based opto-electro-chemical hybrid neural interface.
1.4 Layout of the Dissertation

Chapter 2 summarizes the state-of-art of Optogenetic technology and light delivery strategy. Chapter 3 gives a review of the state of art diamond-based devices and the theory of electrochemistry for chemical sensing. Chapter 4 introduces an SU8 based μLED probe for Optogenetics, which serves as a comparison for evaluating the high thermal conductivity of diamond probe. Chapter 5 reports a hybrid neural interface with a diamond as a heat spreader for optical stimulation and neurophysiological recording. The heat spreading performance is evaluated using a high-resolution infrared camera and compared with an SU8 probe with same dimension and layout reported in Chapter 4. The results show that the maximum temperature of the diamond probe is ~90% lower than that of the SU8 probe. Besides, the functionality of the diamond probe was tested in vivo where light-evoked action potentials were successfully detected. Chapter 6 shows a novel wafer-transfer process of transferring BDD patterns from diamond growth onto a flexible Parylene substrate. The electrochemical properties of the transferred BDD-polymer electrodes are evaluated using (i) an outer sphere redox couple Ru(NH$_3$)$_6^{2+/3+}$ to study electron transfer process and (ii) quantitative and qualitative studies of neurotransmitter redox dopamine/dopamine-quinone. A linear response of the BDD sensor to dopamine concentrations of 0.5 μM to 100 μM was observed ($R^2 = 0.999$) with a sensitivity of 0.21 μA/cm$^2$·μM. Finally, conclusions and future works are given in Chapter 7.
Chapter 2. A review of Optogenetics and light delivery methods

2.1 Microbial Opsins

The core components used in Optogenetics are light-sensitive microbial opsin. There are four major types of opsins: Channelrhodopsin (ChR), Halorhodopsin (HR), Bacteriorhodopsin (BR) and Opsin-receptor chimaeras OptoXRs as demonstrated in Figure 2-1. Channelrhodopsins are light-activated cation channels. The direction of net photocurrent due to ChR activation is down the electrochemical gradient, which polarizes membranes and enables action potentials. Halorhodopsin (HR) is a chloride pump, which pumps chloride ion from extracellular into intracellular space. Similar to Halorhodopsin (HR), Bacteriorhodopsin (BR) is a proton pump, which pumps protons from cytoplasm to extracellular medium. Both Halorhodopsin (HR) and Bacteriorhodopsin (BR) hyperpolarizes membranes and inhibits neural activities. OptoXRs refers to opsin-receptor chimaeras, which can initiate light-activated G protein-coupled biochemical signaling cascades in targeted neurons. If we categorize the opsins according to the functionality, they can be grouped as excitation, inhibition, bi-stable modulation and modulation of

Figure 2-1 Major classes of single-component Optogenetics tool. (Reprinted from[83])
intracellular biochemical signaling. The details of each functional group will be described in the following sections.

2.1.1 Fast Excitation

Channelrhodopsins (ChRs) were first identified by Nagel et al. in 2002[84], where a protein encoded by one of the genomic sequences from green algae Chlamydomonas reinhardtii show light-modulated ion-flux property. Then the initial demonstration for neuroscience application was done by Boyden et al.[85]. However, there are several limitations for those early stage wild-type ChRs. Firstly, the light spectrum of the early stage ChRs is in the blue light wavelength, which has limited penetration depths into neural tissue as light strongly absorbed and scattered compared with higher wavelength lights such as yellow – red wavelengths [86]. Secondly, the wide-type ChRs do not have fast enough off-kinetics to achieve reliable spiking above 40Hz, which is required by many neuronal cell types and physiological processes[83]. Lastly, wild-type ChR2s have relative small photocurrent. The evoked photocurrent under illumination is the current for depolarizing the neurons, which depends upon many factors, including the properties of the opsin being expressed, the wavelength, intensity and duration of the incident light, and even recent illumination history[83], [87]. Small photocurrent requires high light intensity to evoke reliable action potentials, especially when stimulating through thick layers of tissue[88]. To address these limitations, the molecular modification has been extensively used for engineering ChR to achieve certain desired improvements of opsins such as larger peak activation wavelength, faster off-kinetics or higher photocurrent. To date, most of ChR used for Optogenetics have been molecular modified and those variants with different kinetic and spectral attributes are summarized in the blue circle of Figure 2-2. Although the
successful improvement introduced by gene mutation, often times, an improvement of one aspect of the opsin will impair other aspects of the opsin. For example, the introduction of H134R to ChR2 can increase photocurrent magnitude by 2-fold at the expense of slower channel closure kinetics by 2-fold, which gives a poorer temporal precision[89].

Enormous interests have been shown in the development of a red-shifted opsin that can manipulate two isolated neuronal populations with either pre-existing ChR2 or red-shifted opsin expressed under the same volume of tissue. Such opsin requires having a red-shift of more than 50nm on the peak activation wavelength to that of ChR2 in order to avoid any interferences or crosstalk between each population. However, current molecular modification of ChR2 can only have red-shifts of ~30nm, which is between the safety spectrum separations. Zhang et al.[90] discovered an opsin (VChR1) from Volvox carteri., which has ~75nm red-shift to ChR2 and sufficient to achieve independent manipulation. However, the VChR1 expressed neurons show low expression in mammalian neurons with small photocurrents. Then a new family of chimaeric red-shifted opsins was created by Yizhar et al.[91], which comprises of sequences of ChR1 and VChR1 fragments and has a peak activation wavelength between 535 to 545 nm. ChR1/VChR1 chimaeras (C1V1) show enhanced expression and photocurrent in HEK cells compared with only VChR1 expressed cells. A summary of VChR1, chimaeras C1V1 and its variants with different peak activation wavelength and kinetics is shown in the green circle of Figure 2-2.

2.1.2 Fast Inhibition

Above mentioned excitatory opsins like ChR2 can only test the sufficiency of the contribution of the targeted cells to the neural circuitry or behavioral property[92]. However, multiple different cell populations could be involved and give rise to the same
consequences. An ideal solution to test the necessity of targeted cell is to complement excitatory opsins with inhibitory opsins to permit both excitation and inhibition to the targeted neural population with independent light control[92]. Light-driven chloride pump - bacteriorhodopsin and proton pump - Halorhodopsins are two types of opsins that homologues to ChR2 and proved to be capable of inhibiting neural activity.

An initial study of chloride pump was conducted by Zhang et al.[93], which focused on Natronomonas pharaonic (NpHR) and Halobacterium salinarum (HsHR). Both NpHR and HsHR expressed cells lead to rapid outward currents under illumination of light with maximum activation wavelength of 580nm, which is red-shifted enough from the maximum wavelength of ChR2, 460nm. This gives the capability of activation independently of excitatory and inhibitory opsins. Theoretically, a chloride pump should have a low affinity on the intracellular regime for neuron activity inhibition, where Cl$_2$ ions are released. Further investigation found that HsHR has a lower extracellular Cl$_2$ affinity and showed rapid rundown of current at low extracellular Cl$_2$ concentration and does not recover without light illumination. On the contrary, NpHR showed higher extracellular Cl$_2$ affinity and stability and was chosen as inhibitory opsins for optical inhibition study.

Chow et al. (Mac, Arch) [7] and Gradinaru et al. (eBR)[94] explored the use of proton pumps as Optogenetic tools for neural activity inhibition. Opsin archaerhodopsin-31 (Arch)[7] from Halorubrum sodomense can achieve almost 100% silencing of neurons in the awake mouse cortex under yellow-green light illumination (566nm) and can mediate current up to several hundred pico-amps with low light intensity and shows very fast kinetics of recovering from light-dependent inactivation. Opsin Mac[7] from fungus Leptosphaeria maculans provides an opportunity to silence neural activity using blue light,
which enables the potential possibility of silencing two neural populations with different colors of light (blue versus red). Opsin eBR[94] is adopted from BR for optimal membrane trafficking, which can deliver 50pA of outward photocurrent and 10mV hyperpolarizations with optimal wavelength light of 560nm, which is sufficient to silence spiking in the hippocampal pyramidal neuron. A summary of fast inhibition opsins with different peak activation wavelength and kinetics is shown in the red circle of Figure 2-2.

2.1.3 Step-function opsin (SFO)

Step function opsins are a group of ChR mutants that the photocurrents of which can be precisely turned on and off with different colors of light. The SFO opsins have vastly longer time constants than the wild-type ChR2. For example, the time constants of C128T, C128A, and C128S mutants are 2s, 42s, and 100s[95], compared with wild-type ChR2 of ~10ms. With the additional and combinatorial mutagenesis of these early SFO opsins, Yizhar et al.[91] reported stabilized SFOs with time constant of 30min (mutant 128S/156A), which can be used to “step” targeted neurons to a stable depolarized potential with blue light (470nm), followed by removing the light source and starting behavior or physiological study. Then, the SFOs can be deactivated with a yellow light pulse (590nm) illumination. The benefit of SFOs is to rule out the photocurrent artifacts caused by light illumination, which always mingle with the recorded neural signals upon optogenetic stimulations. Besides, ChRs with SFO mutants is responsive to the light intensity at least 300-fold lower than the wild-type ChR[95]. This can lead to excite a larger population of neurons at a given light intensity[83] and prevents the potential tissue over – heating effect by the light sources[83]. A summary of SFO and its variants with different peak activation wavelength and time constants is shown in the pink circle of Figure 2-2, where the
activation wavelength of ChR2 SFO and VChR1-SFOs are 470nm and 560 nm, respectively and deactivation wavelength are 590nm and 390 nm, respectively[95], [46], [91].

2.1.4 Biochemical Modulation

The microbial opsin genes described above are defined as type I, which primarily modulate ion flow to control the excitation or inhibition of a neuron by manipulating the membrane potential to either depolarize or hyperpolarize the cells. Another type of opsin – vertebrate rhodopsin, defined as type II, is a tool for modulating intracellular biochemical signaling. Vertebrate rhodopsin is both a type II opsin and a G protein-coupled receptor (GPCR), which can modulate the G protein signaling on the intracellular side by adsorption of photons. Optogenetic modulation of biochemical signaling can be achieved by constructing chimeras[96], which are referred to as OptoXRs (opsin–receptor chimaeras). In those OptoXRs, the intracellular loops of vertebrate rhodopsins are replaced with conventional ligand-gated GPCRs of the host cells, such as dopaminergic, serotonergic and adrenergic receptors for optical control of intracellular signaling in freely moving mice[97]. The engineering tools designed for type I opsins can be adapted into type II opsins for biochemical signaling. Airan et al.[97] reported Opto-β2AR and Opto-α1AR chimaeras for modulating adrenergic Gs-protein signaling and Gq-protein signaling, respectively with a laser diode-coupled fiber optic devices in nucleus accumbens. A Rh-CT(5-HT1A) chimaeras was published by Oh et al.[98] as a suitable proxy of agonist-induced 5-HT1A receptor activation, which is linked to G<sub>i/o</sub> signaling pathway upon light stimulation.

Besides, microbial photoactivated adenylyl cyclase (AC) shows low cyclase activity in darkness but much higher in the light, which catalyzes adenosine triphosphate (ATP) to
3’,5’-cyclic AMP (cAMP) and pyrophosphate. The cAMP produced by AC is then served as a second messenger for intracellular signaling. Therefore, by manipulating light illumination, photoactivated AC can be used to modulate the activity of the second message in the intracellular signaling. Stierl et al. [100] published that photoactivated adenylyl cyclase (bPAC) mediate light-dependent cAMP increases in Drosophila central nervous system. A Blac gene encoded photoactivated adenylyl cyclase was designed by Ryu et al. [101] with blue light sensitivity to modulate second cAMP and cGMP levels in vivo. A summary of as mentioned chimaeras and photoactivated AC is shown in Figure 2-2 as highlighted in orange.

![Figure 2-2 Kinetic and spectral attributes of Optogenetic tool variants. The variant refers to ChR2 mutation if not specified (Reprinted from [99])](image)

### 2.2 Optogenetic Neural Implants

In order to fully realize the remarkable potential of these opsins, engineering tools for simultaneous light delivery and electrophysiological recording is needed. For *in vitro* light
delivery, in 2005, Boyden et al.[102] demonstrated reliable, millisecond, single-component, genetically targeted optical neuromodulation, where ChR2-expressing hippocampal neurons were excited using an incandescent lamp (450–490 nm, 300W) with a chroma excitation filter, and the light-induced neural activity was recorded using a whole-cell patch clamp. Following that, Ishizuka et al.[103] utilized a surface-mounted, blue-light-emitting diode (LED) (470–490 nm) to quantify the relationship between the light-gated current and the intensity of blue light illumination on ChR2-expressing hippocampal cell cultures. Other in vitro optical instruments have also been reported, such as a focused laser beam using acousto-optic deflectors[104] and digital micro-mirror devices (DMDs)[105],[106]. Although these in vitro approaches can successfully activate neural activity in both cultured neuronal and acute slice preparation, they are not suitable for in vivo stimulation in the intact brain or for study in freely behaving animals.

The first demonstration of functional optical control of intact animal brains was reported in 2007 by Dr. Deisseroth’s group [107]. In their studies, the motor cortex of living rodents was stimulated through an intracranial, multimode, optical fiber coupled to a solid-state laser diode system, with an output light intensity of ~380 mW/mm². Since then, many implantable light delivery systems have been implemented by coupling a thick optical fiber of a few hundred microns to a laser or LED light source. Such systems have been used to study the light-evoked neural activity as well as behavioral changes in commonly used animal models, both small (mice/rats) [108],[109],[110],[111],[112] and large (non-human primates) [113],[114], [115], [116]. These systems, however, inevitably activate many uninterested neurons and are impractical in the spatial control of multi-site stimulation in large-scale neural networks. Therefore, there has been an increased need for the development of implantable, reliable light delivery and recording interfaces with high spatiotemporal resolution and spectral control ability[83].
Recently, advanced microfabrication techniques have been investigated to construct and miniaturize optical neural implants capable of multi-site, localized light stimulation of three-dimensional (3D) brain networks with fine spatial resolution. These devices can be categorized into two major groups based on different light sources: laser, including laser diodes and diode-pumped solid-state (DPSS) laser diodes, and LEDs, including bulk LEDs and microscale LEDs (µLEDs). Optical fibers, microwavesguides, channel waveguides, and tapered optrodes are most commonly used to guide light from sources to target neurons. Microfabricated probes with µLEDs mounted directly at the tip of the probe shaft have also been implemented by several groups. Furthermore, monolithic integration of miniaturized optical elements with multi-electrodes and wireless interfaces enables spatially-confined optical stimulation and simultaneous recording of light-evoked neural activity in freely moving animals.

This section reviews some of the representative microimplants for Optogenetic applications and their related fabrication technologies. Section 2.2.1 summarizes microscale optical implants based on lasers or laser diodes. Section 2.2.2 is devoted to microimplants based on LED light sources.

2.2.1 Laser-coupled Optical Neural Implants

Effective photostimulation of Optogenetic opsins requires the minimum irradiance of 1 (or 7) mW/mm² for neural excitation (or inhibition)[117]. The practical requirement of irradiance is also affected by the high degree of light scattering and absorption in neural tissue[83]. For these reasons, fiber-coupled lasers with high power are being widely used as light sources for many Optogenetic experiments[109][117][87][118][119]. A laser can generate coherent light with unique characteristics: low divergence to focus the light beam over a long distance and high temporal coherence on confining the bandwidth of emitted
light within a narrow spectrum. The former characteristic allows light to be steered through optical fibers to target cells with lower loss than with incoherent light sources (e.g., LEDs). This results in more efficient coupling between light sources and fibers with thin core diameters of 50 microns or less. The latter characteristic enables the high efficiency of optical stimulation since the majority of irradiance will fall into the peak activation spectra of microbial opsins and contribute to optical stimulation. To deliver laser light into target cells, waveguiding structures must be used and are typically implemented by several fabrication techniques, including glass-sharpened optical fibers, out-of-plane microwaveguide arrays, and in-plane microwaveguide probes. In the following sections, I will discuss the device configurations and fabrication techniques of different laser-coupled, optical neural interfaces. Representative prototypes are presented in Figure 2-2, and their specifications are summarized in Table 2-1 [76], [120], [121], [122], [123], [124], [125], [107], [109], [118], [126], [127].

2.2.1.1 Glass-sharpened optical fibers

Single site glass-sharpened optical fibers are typically made of commercially available multimode optical fibers with core diameters of ~200µm. To reduce the thickness of a multimode fiber for localized optical stimulation, in some approaches the plastic cladding layer of the fiber was stripped and the bare glass core with a minimum diameter of 100 µm was guided into a rodent brain through an implanted cannula[128][87]. Wet chemical etching is often employed to sharpen the tip of the glass core in order to further improve spatial resolution and minimize the tissue damage during device insertion. Figure 2-2(a) shows combine a multimode optical fiber attached with four tetrode bundles for electrophysiological recording[109]. Figure 2-2(b) shows a dual-core optical fiber system.
One optical core for optical stimulation and one hollow core filled with 1-3M NaCl for electrical recording[119].

2.2.1.2 Out-of-plane microwaveguide arrays

These devices are normally micromachined, employing thin out-of-plane waveguide shanks with tapered tips to improve spatial resolution and reduce implant invasiveness. The light illuminated by laser light sources is butt-coupled to the waveguide shank and then emitted from the tip for neural stimulation. The optical waveguides can be readily integrated with silicon Utah multielectrode probes for simultaneous stimulation and recording of neural activity. One such device is a SiO$_2$ Utah waveguide array capable of optical stimulation with both visible and infrared (IR) light. This device consists of 10×10 arrays of optrodes 0.5 mm to 2 mm long at a 400 µm pitch, constructed by bulk micromachining fused silica or quartz dices of 3 mm thickness and 50 mm diameter. A dicing saw with a bevel blade was used to shape the pyramidal tips with a precisely controlled taper slope[127]. Furthermore, Zhang et al. reported a dual-modal optrode array[126][129][130][131] (Figure 2-2(c)) modified from a previously developed silicon Utah multielectrode array. In their design, one of the 100 silicon shanks was replaced with a multimode optical fiber, by removing a shank, drilling a hole using ablative laser machining, inserting the fiber through the hole mechanically, and then bonding the fiber with adhesive epoxy.
Figure 2-3 Examples of laser-based optical neural interfaces: (a) A dual-core optical fiber system with one optical core for optical stimulation and one hollow core filled up with 1-3M NaCl for electrical recording. (Reprinted from [67]) (b) A multimode optical fiber with four tetrode bundles attached for electrophysiological recording. (Reprinted from [69]) (c) A dual-mode optrode array adapted from a Utah multielectrode array, where one recording shank was replaced with a multimode optical fiber. (Reprinted from [72]) (d) An in-plane neural probe adapted from conventional Michigan neural probe with embedded dielectric waveguides and microfluidic channels. (Reprinted from [79]) (e) A 3D multiwaveguide array consisting of a set of waveguide combs assembled on a base plate-holder through two alignment and fixation pieces. (Reprinted from [81])
Table 2-1 Summary of the specification of miniaturized, laser-based Optogenetic neural implants

<table>
<thead>
<tr>
<th>Light source</th>
<th># of channels</th>
<th>Dimensions</th>
<th>Output light intensity (max. or used)</th>
<th>Light delivery efficiency</th>
<th># of channels</th>
<th>Dimensions</th>
<th>1kHz Impedance</th>
<th>Other capabilities</th>
<th>Substrate material</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical fiber coupled waveguide (Oxynitride core)</td>
<td>1</td>
<td>70µm wide</td>
<td>7mW/mm²</td>
<td>-10.5 ± 1.9 dB</td>
<td>8</td>
<td>143µm in diameter 20µm separation</td>
<td>1.37MΩ</td>
<td>No</td>
<td>Si</td>
<td>[76]</td>
</tr>
<tr>
<td>Bare Laser chip coupled waveguide (SU8 core)</td>
<td>1×2</td>
<td>15 µm wide 13µm long @ 659nm</td>
<td>29.7mW/mm²</td>
<td>--</td>
<td>2×4</td>
<td>20µm in diameter</td>
<td>1.54± 0.06MΩ</td>
<td>No</td>
<td>Si</td>
<td>[120]</td>
</tr>
<tr>
<td>Optical fiber coupled waveguide (SU8)</td>
<td>1</td>
<td>0.15mm in width</td>
<td>60mW/mm²</td>
<td>-12dB</td>
<td>8</td>
<td>--</td>
<td>280KΩ - 350KΩ</td>
<td>No</td>
<td>Micro-fluidic Channel</td>
<td>Polyimide</td>
</tr>
<tr>
<td>Optical fiber coupled waveguide (SU8)</td>
<td>1</td>
<td>≤150µm wide</td>
<td>0.9mW</td>
<td>--</td>
<td>16</td>
<td>20µm×20µm</td>
<td>0.8MΩ</td>
<td>Micro-fluidic Channel</td>
<td>Si</td>
<td>[122]</td>
</tr>
<tr>
<td>Optical fiber coupled multi-waveguide (Oxynitride core)</td>
<td>12</td>
<td>60–360µm wide 1cm long 1mm separation</td>
<td>--</td>
<td>-10dB</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>No</td>
<td>Quartz</td>
<td>[123]</td>
</tr>
<tr>
<td>Tapered optical fiber with multi-openings</td>
<td>Max. 7</td>
<td>600nm in diameter</td>
<td>3.5mW</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>No</td>
<td>-</td>
<td>[124]</td>
</tr>
<tr>
<td>Laser-coupled fiber</td>
<td>1</td>
<td>200 µm in diameter</td>
<td>~380mW / mm²</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>No</td>
<td>--</td>
<td>[107]</td>
</tr>
</tbody>
</table>
Table 2-2 (cont’d)

<table>
<thead>
<tr>
<th>Laser-coupled device</th>
<th>ID</th>
<th>Dimensions</th>
<th>Power Density/Intensity</th>
<th>Transmission</th>
<th>No. of Contacts</th>
<th>Type</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Laser-3-D waveguide  | 192| 9µm×60µm   | #1 scheme: 148±56mW/mm²  
|                       |    |            | #2 scheme: 200mW/mm²     |              |                |      | [125]     |
| Laser-coupled fiber  | 1  | 200 µm in diameter | 60~160mW/mm² @ 473nm  
|                       |    |            | 160~260mW/mm² @ 561nm: |              | 4              | Diameter ~25µm | No       | --     | [109]     |
| Laser-coupled fiber  | 1  | Tip diameter of 6-20µm  
|                       |    |            | Fiber diameter of 4 µm |              | 1              | 0.7µm in diameter | No       | --     | [118]     |
| Laser-coupled optrode| 1  | 50-62.5µm in diameter | 916mW/mm² | -1.55dB | 99                 | 1mm long 400µm separation | 112KΩ - 671KΩ | No | -- | [126]     |
| Laser-coupled optrode| 100| 0.5-2mm long 150µm wide 400µm separation | -- | -1.49dB | 0 | - | - | No | SiO₂ | [127]     |
2.2.1.3. In-plane microwaveguide probe

Compared to the out-of-plane arrays, in-plane microwaveguide probes benefit more from modern microelectromechanical system (MEMS) technology evolved from the process technology in the conventional semiconductor device fabrication. Most of these probes share a similar configuration: an in-plane microwaveguide for light delivery carried by a silicon or polymer shaft with electrophysiological recording and/or microfluidic modalities. Several combinations of dielectric materials used for microwaveguides include: oxynitride core (refractive index: 1.51) with oxide clad (refractive index: 1.46)[132], and SU-8 core with either silicon oxide[120], tungsten-titanium alloy (10% titanium)[121], or glass clad[122]. For the two designs with microfluidic modality, integrated microchannels are constructed by either photopatterning of SU-8[121] or reflow of borosilicate glass followed by chemical mechanical polishing (CMP)[122] (Figure 2-2(d)). Light coupling between laser light sources and planar microwaveguides is typically achieved through optical fibers.

Despite their significant advantages, the aforementioned devices are limited to delivering light to a single target, and therefore not suitable for applications that require delivering patterned light independently to distributed targets in 3D brain circuits, such as in the rhesus macaque cortex[123]. From a fabrication perspective, a straightforward approach to increase the spatial density of optical stimulation is to assemble 3D arrays with planar, multi-shank waveguide probes, using possible methods originally developed for 3D Michigan type multielectrode arrays. Such methods include backbone stacking and bonding[133], folded Parylene cable[55], and orthogonal insertion of planar probes into a carrying platform[134]. Making planar waveguide probes with multiple shafts can be achieved simply by modifying
2.2 LED-Based Optical Neural Implants

Although lasers and laser diodes provide several benefits, including high light intensity, low-beam divergence, and narrow spectral bandwidth, laser-based optical systems have the following drawbacks. First, lasers are power hungry with typical power consumption of several tens of mW per channel. Second, when used with freely behaving animals, lasers require the use of tethered optical fibers and commutation systems, which greatly restrict the natural behavior of the subjects. Finally, the complexity of device fabrication may lead to increased costs due to the requirements of multiple waveguides and light sources. To expand the spatial resolution into three dimensions, a 3D multiwaveguide array [13] was implemented, consisting of a set of waveguide comb arrays inserted orthogonally into a base plate holder with the assistance of two alignment and fixation pieces. These devices, while successfully demonstrated, still have a large footprint along a single probe shaft. As the waveguides can be separately coupled to different light sources, this device enables independently addressable optical stimulation at each output with adjustable wavelength. To further increase spatial resolution, a 3D multiwaveguide array [13] was reported by Zorzos et al. [123]. In this approach, twelve varying-length dielectric microwaves were lithographically patterned on the same shaft with independently addressable optical stimulation at each output with adjustable wavelength. To expand the spatial resolution into three dimensions, a 3D multiwaveguide array [13] was implemented, consisting of a set of waveguide comb arrays inserted orthogonally into a base plate holder with the assistance of two alignment and fixation pieces. These devices, while successfully demonstrated, still have a large footprint along a single probe shaft. As the waveguides can be separately coupled to different light sources, this device enables independently addressable optical stimulation at each output with adjustable wavelength. To expand the spatial resolution into three dimensions, a 3D multiwaveguide array [13] was implemented, consisting of a set of waveguide comb arrays inserted orthogonally into a base plate holder with the assistance of two alignment and fixation pieces. These devices, while successfully demonstrated, still have a large footprint along a single probe shaft. As the waveguides can be separately coupled to different light sources, this device enables independently addressable optical stimulation at each output with adjustable wavelength.
power consumption, illumination stability, and fast light-switching ability[19]. More importantly, electronically driven LEDs are particularly suitable for integration with wireless telemetries to enable fully implantable systems for applications in freely behaving animals[20]. A variety of μLED processing approaches has been developed by many researchers, such as Jeon et al.[136], Zhang et al.[137], and Kim et al.[25]. The fabrication of μLED-coupled optical probes relies on two basic stereotypes of neural probes being used for electrical stimulation: Utah-type[138] and Michigan-type[139]. In the following sections, the device configurations and fabrication techniques of different μLED-coupled optical neural interfaces are discussed in detail. Representative prototypes are presented in Figure 2-3 and their specifications are summarized in Table 2-2 [140], [141], [142], [143], [144], [145], [76], [26], [146]

2.2.2.1. Utah-type optical arrays

The Utah neural probes, which were made by bulk micromachining thick boron-doped silicon substrates, have been widely used for electrical stimulation and chronic neural recordings[147],[148]. Compared with the Michigan probe, the Utah-probe topology enables the arrangement of high-density shanks in a 3D configuration. Taking this advantage, the Utah-probe topology has been adopted to make LED-coupled optical probes for Optogenetic applications. Two main designs of Utah-type optical probes include planar, surface-mounted LED arrays and 3D arrays with μLEDs coupled to optical fibers or waveguides. Probes based on the former design are primarily used in in vitro studies with cell culture and brain slice preparations, while the latter design targets in vivo studies in the deep cortical layers and brain regions of living animals. The following two sections will
discuss in more detail the surface-mounted μLED arrays and optical fiber/waveguide-coupled μLEDs array.

2.2.2.1.1 Surface-mounted μLED arrays

Grossman *et al.*[136],[149],[142],[150] (Figure 2-3(a)) used conventional silicon-based microfabrication technology to build the first custom designed, high-power μLED array, which can generate arbitrary optical excitation patterns with micrometer and millisecond resolution. Despite the successful demonstration of optical modulation of neural activity with high density and high spatial resolution, this type of probe suffers from disadvantages related to the integration of neural recording capability and heat generation due to high-density μLED illumination. In the *in vitro* study, because a whole cell patch clamp was used for neural signal recording, it was difficult to record signals from multiple neurons simultaneously. In addition, these μLED arrays would have difficulties in thermal management, especially when operated at high frequency and long duration, due to their ultra-high density (a 64×64 μLED array with a small pitch of 50μm). The excessive heating may cause tissue damage as well as physiological and behavioral change[151], which may bias the outcomes of Optogenetics.

Beside custom made μLED arrays, commercially available die-form LED chips are employed in the fabrication of surface-mounted optical arrays. Polymers, such as Parylene-C, SU-8, and polyimide[43], have been used as carrying substrates and insulating layers of the LED chips, due to the mechanical flexibility, biocompatibility, chemical resistance, and stability of the polymers. As an example, Kwon *et al.*[152],[153] (Figure 2-3(b)) reported a multichannel Opto-μECoG array, which combines a transparent microelectrode array and a μLED array on a flexible Parylene-C substrate for epidural optical stimulation and
electrical recording of cortical activity. The µECoG array featured a Parylene-indium tin oxide (ITO)-gold-Parylene sandwich structure for electrical recording without compromising the optical throughput. The µECoG array and the µLED array were fabricated separately, and then aligned and bonded together using adhesive polymers. It is of note that the maximum temperature variance of this device was ~9 °C and ~1 °C when the LED was driven by 100 ms voltage pulses of 3.2 V and 2.7 V, respectively. Hence, the application of this probe is limited to short pulses with low applied voltage.

2.2.2.1.2 Optical fiber/waveguide-coupled µLED arrays

The surface-mounted µLED arrays have limited stimulation depths due to the scattering and absorption of LED light by neural tissue[151]. In view of such shortcomings, significant developments have been made to couple µLED light into waveguiding structures, such as optical fibers, microwaveguides, and optrodes, for delivering light into deep brain regions. For example, a 3×3, LED-coupled, optical fiber array was reported by Schwaerzle et al.[140],[154] (Figure 2-3(c)). In this design, commercially available µLED were flip-chip bonded onto a polyimide substrate with patterned metal interconnects. DRIE was used to machine a miniaturized silicon housing plate that carried the µLED chips and aligned them to optical fibers with high precision. The equivalent coupling efficiency between LEDs and fibers was measured to be ~ 0.88-1.27%. When the LEDs were driven by 30 mA current pulses, the maximum temperature variations on the silicon housing were 5 °C, 10 °C, and 15 °C, with 5 %, 10 % and 15 % pulse duty cycles.

Recently, LED-coupled SU-8 microwaveguide arrays have been developed by Kwon et al.[141],[155],[156],[157],[158],[159],[160] (Figure 2-3(d)), using polymer-based MEMS techniques. These 3D arrays were equipped with single-length or varying-length slanted
microwaveguides to deliver light to the same or different layers of the cortex. Backside UV exposure of an SU-8 photoresist was used to create taper-shaped waveguides with the tip and base diameters controlled by adjusting the mask designs and the separation between the mask and the resist layer. Building on the 3D waveguide arrays, an ITO-Parylene-gold-Parylene sandwich clay was coated on the outer sidewalls of the SU-8 waveguides to serve several functions. In particular, the ITO layer was used to eliminate photoelectrical artifacts without compromising the light transmission. The opaque gold layer was used to block out the light leakage and record light-evoked neural activity. The coupling efficiency was estimated to be ~10 %, which is close to the maximum coupling efficiency of LED-fiber butt coupling[161]. The maximum temperature variation of the LED was ~ 9 °C with an applied voltage of 3.2 V and 100 ms activation duration.

While the above fiber/waveguide-coupled µLED arrays have been successfully tested in vivo, the main limitation of these devices is the low efficiency of the LED-fiber butt coupling, which is typically less than 10 %. In order to maintain sufficient light intensity for effective photostimulation of opsins, high applied voltage and long duration of driving pulses are necessary, which will inevitably increase the local temperature. The low thermal conductivity of the polymer substrate and package also prevents heat transfer from the devices to the surrounding biological environment, therefore increasing the risk of thermally-induced tissue damage.

2.2.2.2 Michigan-type optical probes

In order to achieve highly efficient light coupling, an alternative light delivery strategy has been explored by inserting µLEDs directly into deep brain targets of interest. Well-established Michigan-type probes provide an ideal platform where both custom designed
and commercially available µLED chips can be mounted onto the probe tip as light sources for optical neuromodulation. Michigan-type optical probes constructed with commercial µLED chips have been reported by many research groups.

Although using commercially available µLED chips can reduce the complexity of device fabrication and µLED assembly, the dimensions of µLEDs are limited by factory specifications, which make it difficult to miniaturize the devices. A custom designed µLED chip will provide an opportunity for the miniaturization of Michigan-type probes to increase the spatial resolution of photostimulation, reduce device invasiveness, and prevent unnecessary tissue damage. Both semiconductor-based and polymer-based microfabrication technologies have been explored. A representative prototype built using traditional semiconductor technology was reported by McAlinden et al.[162],[163] (Figure 2-3(e)). In this case, a blue µLED probe was fabricated from a commercial LED wafer with epitaxial GaN structures grown on a sapphire substrate. A 7-mm-long probe carried five LEDs (with a diameter of 40 µm and a pitch of 250 µm) on a 1.3-mm-long tip shaft for optical neuromodulation. Laser dicing was used to shape the sapphire probe, followed by mechanical thinning of the probe to 100 µm thick from the backside of the substrate. With a 200 ms driving pulse (resulting in 600mW/mm² light intensity), the probe had a maximum temperature rise of less than 2 °C, which benefits from the relatively high thermal conductivity of sapphire (23 W·m⁻¹·°C⁻¹). Despite the low heating benefit, this device suffers from the mechanical rigidity of sapphire, which may promote a neuro-inflammatory response, induce mechanical strains in the surrounding tissue, and lead to irreversible tissue damage during chronic applications in freely moving subjects.

As a solution, mechanically compliant polymeric substrates can be used to carry the
LEDs. However, most fabrication processes for making inorganic LEDs, particularly for blue GaN LEDs, are incompatible with polymers, due to the requirement of high processing temperatures. Therefore, novel techniques are needed to transfer pre-fabricated µLED chips from a solid substrate (sapphire or silicon carbide) to a flexible polymer substrate. Kim et al. [25] demonstrated a µLED substrate transfer technology, by which µLEDs with lateral dimensions ranging from 1mm×1mm to 25µm×25µm can be transferred from a foreign substrate such as sapphire to a target polymer substrate. Using this technology, Kim et al.[26] (Figure 2-3(f)) implemented a flexible, multifunctional, neural interface probe, which consisted of a platinum microelectrode array for neurophysiological recording, a µLED array for optical neuromodulation, a precision temperature microsensor layer for real-time monitoring of local temperature variation, and a microscale, ultrathin silicon photodiode array for measuring the light intensity of µLEDs. Each sensor/array layer was constructed on a flexible polyester substrate, and then stacked and bonded together with UV-curable epoxy. A releasable base was fabricated from epoxy and bonded to the as-fabricated hybrid neural probe with bio-resolvable adhesive silk to facilitate the insertion of the mechanically flexible probe into brain tissue. The time-average temperature change with various duty cycles at 17.7 mW/mm² peak light output was within 1 °C when the device was inserted 0.3mm into brain tissue. As the state of the art, these multifunctional optoelectronics present significant advantages they are not without significant concerns for experiments with freely moving animals. The devices were tested in vivo to demonstrate their functionalities for optical stimulation, electrical recording, and dissecting complex neurobiology and behavior of freely moving animals. The time-average temperature change with various duty cycles at 17.7 mW/mm² peak light output was within 1 °C when the device was inserted 0.3mm
into brain tissue. As the state of the art, these multifunctional optoelectronics present significant advantages, including spatially precise and cellular-scale light delivery, highly effective thermal management, mechanical flexibility, and integration with wireless components to eliminate the need for fiber optics, tethers, and commutators. While such devices have shown great improvements over the previous instruments, they are not without significant concerns. These
Table 2-3 Summary of the specification of miniaturized, µLED-based Optogenetic neural implants

<table>
<thead>
<tr>
<th>Light source</th>
<th># of channels</th>
<th>Dimensions</th>
<th>Output light intensity (max. or used)</th>
<th>Light delivery efficiency</th>
<th># of channels</th>
<th>Dimensions</th>
<th>1kHz Impedance</th>
<th>In vivo or in vitro validation</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>µLED coupled optic fiber</td>
<td>3×3</td>
<td>5mm long, 105µm in diameter, 550µm separation</td>
<td>1.28mW/mm²</td>
<td>-20.56dB – -18.97dB</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>None</td>
<td>[140]</td>
</tr>
<tr>
<td>µLED coupled optic optrode</td>
<td>32</td>
<td>Base size: 300µm, Tip size: 30µm</td>
<td>10mW/mm²</td>
<td>-10 dB</td>
<td>32</td>
<td>Electrode size: 30µm</td>
<td>10~500kΩ</td>
<td>In vivo</td>
<td>[141]</td>
</tr>
<tr>
<td>Surface mounted µLED array</td>
<td>64×64</td>
<td>20µm in diameter, 50µm separation</td>
<td>250mW/mm²</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>In vitro</td>
<td>[142]</td>
</tr>
<tr>
<td>Surface mounted µLED array</td>
<td>3</td>
<td>200µm in diameter, 700µm separation</td>
<td>10mW/mm²</td>
<td>--</td>
<td>16</td>
<td>200µm in diameter, 700µm separation</td>
<td>1~5kΩ</td>
<td>In vivo</td>
<td>[143]</td>
</tr>
<tr>
<td>Penetrating probe with µLED</td>
<td>5×1</td>
<td>1.3 mm long, 80µm wide, µLED diameter: 40µm, 250µm separation</td>
<td>600mW/mm²</td>
<td>2%</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>None</td>
<td>[144]</td>
</tr>
<tr>
<td>Penetrating probe with µLED</td>
<td>1</td>
<td>12mm long, 900µm wide, µLED: 1×0.6×0.2mm³</td>
<td>0.7mW/mm²</td>
<td>--</td>
<td>3</td>
<td>50µm×100µm</td>
<td>--</td>
<td>In vivo</td>
<td>[145]</td>
</tr>
<tr>
<td>Penetrating probe with µLED</td>
<td>1</td>
<td>4.2mm×0.86mm×0.28mm, µLED: 0.55×0.29×0.1mm³</td>
<td>1mW/mm²</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>In vivo</td>
<td>[76]</td>
</tr>
<tr>
<td>Penetrating probe with µLED</td>
<td>1</td>
<td>~400µm wide, 20µm thick, µLED: 50×50×6.45µm³</td>
<td>7mW/mm²/17.7mW/mm²</td>
<td>--</td>
<td>1</td>
<td>20µm×20µm</td>
<td>1MΩ</td>
<td>In vivo</td>
<td>[26]</td>
</tr>
<tr>
<td>Penetrating probe with µLED</td>
<td>2</td>
<td>8×6.8×0.25mm², µLED: 0.55×0.29×0.1mm³</td>
<td>1.5mW/mm²</td>
<td>--</td>
<td>4</td>
<td>120µm in diameter</td>
<td>20~90kΩ</td>
<td>In vivo</td>
<td>[146]</td>
</tr>
</tbody>
</table>
Figure 2-5 A spider schematic compares several main specifications of the laser- and LED-based microdevices surveyed in this paper, in terms of size, density, multiple functions, wireless capability, maximum light delivery efficiency, and light delivery efficiency. The performance is rated on a scale of 1 to 5, with 5 being the best.

include potential thermally-induced tissue damage due to LED heat deposition in the brain as well as electronic failure, particularly for high-density neural implants where microelectronics are in direct contact with large-area brain tissues. Furthermore, the very limited adaptation of this exciting method by other researchers in this field could be mainly due to the labor-intensive and costly process of device fabrication.

As Optogenetic studies have rapidly expanded into in vivo applications using freely
moving animals, there is an increasing demand for the development of untethered optical tools for wirelessly controlled optical stimulation and neural readouts of large-scale brain circuitry. Two major types of optical microdevices based on laser and LED light sources have been surveyed. An overview of the advantages and disadvantages of both major types is summarized using a spider web diagram as shown in Figure 2-4 from the perspectives of size, functionality, maximum light delivery capability, light delivery efficiency, density and wireless capability. The performance is rated on a scale of 1 to 5, with 5 being the best.
Chapter 3.  BDD neurotransmitter detection sensor

3.1 Theory of Electrochemistry

3.1.1 Nernst equation

From a thermodynamic standpoint, the Nernst equation is derived from the standard changes in Gibbs free energy in a chemical reaction. Considering a half-reaction at anode, where \( v_o \) and \( v_R \) are stoichiometric coefficients,

\[
\nu_o O + ne^- \rightarrow v_R R
\]  (3-1)

and another half-reaction at the cathode,

\[
H_2 \rightarrow 2H^+ + 2e^- \]  (3-2)

the cell reaction is then,

\[
vH_2 + v_o O \pi \rightarrow v_R R + vH^+ \]  (3-3)

The free energy is given by thermodynamic, considering \( a_{H^+} = a_{H_2} = 1 \), where \( a_i \) is the activity of the species \( i \),

\[
\Delta G = \Delta G^0 + RT \ln \frac{a_R^{v_R}}{a_o^{v_o}} \]  (3-4)

Since \( \Delta G = -nFE \) and \( \Delta G^0 = -nFE^0 \), where \( E^0 \) is the cell potential at standard conditions. The potential \( E \) (also called half-cell potential) at the anode or cathode electrode can be calculated,

\[
E = E^0 + \frac{RT}{nF} \ln \frac{a_o^{v_o}}{a_R^{v_R}} \]  (3-5)

which is called Nernst equation. The cell potential can be calculated using

\[
E_{cell} = E_{cathode} - E_{anode} \]  (3-6)

However, the \( E^0 \) of above-mentioned Nernst equation is under the standard condition and it is inconvenient to deal with activates under non-standard condition. A solution to this is to introduce formal potential \( E'_e \), where \( \gamma_i \) is the activity coefficient:
\[ E_e^0 = E_e^o + \frac{RT}{nF} \ln \left( \frac{y_o}{y_R} \right) \]  

(3-7)

Then the Nernst equation can be written as

\[ E_e = E_e^0 + \frac{2.3RT}{nF} \log \frac{C_o}{C_R} \]  

(3-8)

3.1.2 Kinetics of electrode reactions

In the previous section, it was shown that at equilibrium, the potential of an inert electrode at anode or cathode in the solution of Redox (O and R) and supporting electrolyte is determined by Nernst equation. At the situation that away from the equilibrium potential, the kinetics of electron transfer is determined by Butler-Volmer equation:

\[ i_{net} = nF A k^o \left( C_o(0, t) \exp \left( \frac{anF\eta}{RT} \right) - C_R(0, t) \exp \left( -\frac{(1-\alpha)nF\eta}{RT} \right) \right) \]  

(3-9)

where \( i_{net} \) is the net current of forward and backward reaction (A), \( n \) is the number of electrons involved in the electrode reaction, \( F \) is Faraday’s constant (C mole e\(^{-1}\)), \( R \) is universal gas constant(J mol\(^{-1}\) k\(^{-1}\)), \( T \) is absolute temperature (k), \( \alpha \) is the transfer coefficient (dimensionless), \( C_o(0, t) \) is the concentration at distance x=0 from the surface at time t (mol/cm\(^3\)), \( \eta \) is the overpotential (v) and \( k^o \) is the standard rate constant (cm/s). The standard rate constant is a measure of the kinetic facility of a redox couple. A system with large rate constant will achieve equilibrium faster than a system with smaller rate constant.

3.1.3 Mass transport

Mass transport phenomenon describes the supply of reactant and the removal of the product from the electrode. There are three forms of mass transport: Diffusion, Migration, and Convection. Diffusion describes the movement of a chemical species due to the concentration gradient. Diffusion will move the species from high concentration regions to low concentration regions. Migration is the movement of charged species in the presence of an electric gradient. Migration is
an electrostatic phenomenon and not the focus of this thesis. In the experiment, high concentration supporting electrolytes will be used to avoid the influence of the migration in an electrochemical cell. Convection refers the movement of a chemical species due to external mechanical forces. Convection may be introduced by stirring the solution or rotating disk electrode. The mass transport is determined by Nernst-Planck Equation:

\[ J_x = -D_x \left( \frac{\partial C_x(x)}{\partial x} \right) - \frac{z_x F}{RT} D_x C_x \left( \frac{\partial \phi(x)}{\partial x} \right) + C_x \nu(x) \] (3-10)

where \( J \) is the flux of the species (mol cm\(^{-2}\) s\(^{-1}\)), \( D \) is the diffusion coefficient of the solution species (cm\(^2\)/s), \( C \) is the concentration of the species (mol/cm\(^3\)), \( \phi \) is the electrostatic potential (v) and \( \nu(x) \) is the fluid velocity, cm s\(^{-1}\), \( z \) is the charge of species, \( F \) is Faraday’s constant (C mole e\(^{-1}\)), \( R \) is gas constant value (J mol\(^{-1}\) k\(^{-1}\)), \( T \) is temperature (k). The three terms of Nernst-Planck Equation describes diffusion, migration and convection, respectively.

3.2 Techniques for study of electrode reactions

In this section, basic electrochemical techniques will be introduced for studying electrode reactions. The mass transfer process of the systems considered in this section will occur only by diffusion. An excess of the inert electrolyte is added so migration does not contribute to the mass transfer process. No rotating disk electrode or stirring is applied to prevent any convection. The techniques can be categorized into controlled potential technique (potential step) and potential sweep technique. Both controlled potential method and potential sweep method share a same block diagram of measurement as shown in Figure 3-1. A potential step is applied for a controlled potential technique between a working electrode (WE) and a reference electrode (RE) and measures the current flow from working electrode to counter electrode (CE). The recorded current is a combination of Faraday current and double layer charging current.
Figure 3-1 Simplified block diagram for potentiometric measurements.

3.2.1 Controlled potential methods (Potential step)

A demonstration of applied potential and recorded current is shown in Figure 3-2. Potential step experiments often use to obtain quantitative data when a redox system is understood qualitatively by a potential sweep technology such as cyclic voltammetry(CV). This kind of experiment is also called chronoamperometry since the current is recorded as a function of time.

The recorded Faraday current follows Cottrell equation:

\[ i_F(t) = \frac{nFAD_o^{1/2}C_o^*}{\pi^{1/2}t^{1/2}} \]  

(3-11)

where \( i_F(t) \) is measured Faraday current (A), \( n \) is the number of electrons involved in the electrode reaction, \( F \) is Faraday’s constant (C mole e\(^-\)), \( A \) is the area of the planar electrode (cm\(^2\)), \( C_o \) is the bulk concentration of the analyte (mol/cm\(^3\)), \( D_o \) is the diffusion coefficient of the species (cm\(^2\)/s), \( t \) is the time (s). The double-layer charging current caused by double-layer capacitance is determined as below:
\[ i_c(t) = \frac{E}{R_s} e^{-t/R_s c_d} \]  

(3-12)

, where \( E \) is applied potential (V), \( R_s \) is the solution resistance (Ω) and \( c_d \) is the double layer capacitance (F). If the electrode is a microelectrode arrays, where the diffusion layer thickness for each array electrode is less the spacing between each electrode, the Cottrell equation is written as:

\[ i_F(t) = nFAD\left[\frac{1}{(nDt)^\frac{1}{2}} + \frac{1}{r_o}\right] \]  

(3-13)

, where \( r_o \) is the radical distance from the electrode center. Therefore, \( \lim_{t \to \infty} i_F(t) = nFAD/r_o^* \), the recorded current reaches a steady-state condition.

Figure 3-2 A demonstration of applied potential (a) and recorded current (b) for potential step technique. (Figures are adopted from www.studyblue.com)
3.2.2 Potential sweep methods

The complete electrochemical behavior of a Redox system can be studied by applying a series of potential steps and recording the corresponding current-time curves. One of such methods is described is called cyclic voltammetry (CV), as shown in Figure 3-2, where a saw-tooth wave is applied between WE and RE of a chemical sensor and the current-time curve is recorded between WE and CE. The voltammogram has one oxidation peak potential $E_{pa}$ and one reduction peak potential $E_{pc}$ with corresponding oxidation peak current (anodic current) $i_{pa}$ and reduction peak current (cathodic current) $i_{pc}$, respectively. The recorded current contains both background current (double-layer charging current) and Faraday current. For a reversible system (kinetic fast system), the peak current contributed by Redox reaction under semi-infinite linear diffusion condition can be determined by

$$i_p = (2.69 \times 10^5) n^2 AD_0^{1/2} C_0^* \nu^{1/2}$$  \hspace{1cm} (3-14)$$

where $i_p$ is the peak Faraday current (A), $n$ is the number of electrons involved in the reaction, $A$ is the area of the planar electrode (cm$^2$), $C_0^*$ is the bulk concentration of the analyte (mol/cm$^3$), $D_0$ is the diffusion coefficient of the species (cm$^2$/s), $\nu$ is scan rate (V/s). The peak double layer charging current can be determined by

$$|i_c| = A c_d \nu$$  \hspace{1cm} (3-15)$$

where $c_d$ is the double layer capacitance (F). The difference between peak potential $E_p$ and half peak potential $E_{p/2}$, where the peak current is reduced by half, can be used to determine whether a Redox system is reversible or not. For a kinetic fast system (reversible system),

$$|E_p - E_{p/2}| = 2.20 RT/nF$$  \hspace{1cm} (3-16)$$

which equates 56.5/n mV at 25°C.
3.3 Polycrystalline diamond deposition and characteristics

Diamond and graphite are both allotrope of carbon. At room temperature and pressure, graphite is the stable allotrope and it is more stable than diamond, which is the metastable allotrope. Therefore, nature diamond occurs at depths of around 200km down to the earth, where the pressure and temperature are in the range of 70-80 kbar and 1400-1600°C, respectively[164], [165]. The first industrialized diamond synthesis methodology was high pressure, high temperature (HPHT) method proposed by Bundy in 1955 [166]. Later, the chemical vapor deposition (CVD) technology was introduced into diamond synthesis, which enables the processes to happen at low pressures. In this case, diamond growth is driven by kinetics in a high growth temperature rather than
Figure 3-4 A diagram of diamond CVD reactor: (a) hot filaments CVD reactor (b) Microwave plasma CVD. (Figure 3-3 (a) is adopted from [169], Figure 3-3 (b) is adopted from [170].

thermodynamics[59]. The industrialized processes of CVD diamond were reported in the 1980s[167]. The CVD growths rely on a very small fraction of carbon (typical <5%) and relatively high concentrations of dissociated hydrogen (H) with a growth temperature greater than 700°C to provide surface kinetics[168]. During CVD, a plasma can be created by two major methods: hot filaments (HF)[169] or microwaves (MW)[170]. Reactors based on both methods are shown in Figure 3-4 (a) and (b). MW-CVD can generate higher plasma temperatures than HF reactors, which results in the higher concentration of H atoms and can achieve higher phase purity and faster film growth. The plasma temperature generated by HF-CVD is limited by the melting point of the filaments and typically lower than the MW-CVD, but can grow over the larger surface area[168].

The gaseous form carbon source such as methane and dopant, such as B₂H₆ is fed into the CVD chamber with hydrogen (H₂) atmosphere. The presence of H₂ is essential to react with sp and sp² carbon on the surface and convert them to sp³ carbon[171]. Diamond grown by CVD can be divided into two classes — polycrystalline and single crystalline diamond. Polycrystalline diamond is composed by tightly-bonded small single crystals (grains). The grain size of
Figure 3-5 (a) schematic (b) scanning electron microscopy (SEM) image of the cross section of diamond film of MC BDD. SEM imagines of (c) diamond nucleation side and (d) diamond growth side. (a) and (b) are adopted and reprinted from [168]. (c) and (d) are taken using the films and devices reported in Chapter 6.

Polycrystalline diamond is determined by the process parameters such as temperature, pressure, hydrogen to carbon and boron ratios and additional argon gas etc. [172] and can be categorized into ultra-nanocrystal line (UNC) < nanocrystalline < microcrystalline based on grain size. For fixed growth conditions, as grown time increases, a high density of nucleation sites first formed on the substrate and then the sites with favored facets and orientations grow preferentially to those with less favored ones, which results in an increasing size of crystalline facets as demonstrated in
Figure 3-5 (a) and (b)[168]. This will further lead to a smoother surface on the diamond nucleation side than the diamond growth side as demonstrated in Figure 3-5 (c) and (d). The SEM imaging of diamond nucleation and growth side are taken from the boron-doped polycrystalline diamond (BDD) thin film described in Chapter 6. Non-diamond carbon (NDC) can be found predominately at grain boundaries. UNC and NC BDD have more NDC than MC BDD due to more dense grain boundaries[173]. The impurity caused by NDC of polycrystalline BDD film can cause the electrode less electrocatalytically inert. Electrochemically, this can lead to a reduced potential windows, increased background current and a surface more susceptible to fouling. Besides, in the electron transfer process, a higher impurity of NDC results in slower electron transfer kinetics for certain inner sphere redox couples. A comparative study of the potential window and background current among diamond nucleation side, diamond growth side and a commercially available gold electrode in 1M KCL solution with Pt as CE and Ag/Agcl as a reference at a scan rate of 0.1V/s is shown in Figure 3-6. Those data are taken from the BDD film reported in Chapter 6. The voltammograms are taken before and after the substrate transfer, which gives both diamond growth side and nucleation side.

The easiest way to measure the impurity of diamond (primarily sp² carbon) is to use Raman spectroscopy. A pure sp³ diamond has only σ bonds, which results in a characteristic peak at 1332 cm⁻¹. The line width of the peak is a qualitative measure of defects in the diamond film. The wider the line width, the more defects. NDC, primarily sp² carbon, which contains π bonds gives another two peaks at 1355 cm⁻¹ (D peak) and 1575 cm⁻¹(G peak)[174]. The ratio of the relative intensity of the sp³ peak to sp² G peak is used to assess the quality of the diamond film in terms of non-diamond carbon impurity. Since π bonds are more polarizable than σ bonds, the excitation wavelength of Raman spectroscopy is very important to accentuate the features of interest. Popular
laser wavelengths for Raman spectroscopy are 514.5nm green Ar ion laser, 632 nm HeNe laser, and 785 nm IR diode laser. The longer wavelength of the excitation laser, the more sensitive to sp² carbon[168]. Figure 3-7 shows Raman spectroscopy of heavy boron doped polycrystalline diamond with different B/C ratio in the gas phase[175]. For boron-doped diamond, the ‘1332 cm⁻¹’ peak decreases and down-shifts as B/C ratio (Boron to Carbon ratio) increases. Two new peaks at 500 cm⁻¹ and 1220 cm⁻¹ appear. The relative intensities of both new peaks increase as boron

Figure 3-6 Voltammograms of Au, BDD growth side and nucleation side in 1M KCL solution (WE: BDD / Au, CE: Pt, RE: Ag/Agcl), Scan rate: 0.1V/s. This experiment is done using the films and devices reported in Chapter 6.
Figure 3-7 Raman spectroscopy of heavily boron doped polycrystalline diamond with different B/C ratio in the gas phase. Reprinted from [175]

content increases in the gas phase. Two new broad peaks at 1350 cm\(^{-1}\) and 1580 cm\(^{-1}\) appear for very high B/C ratio (14000ppm), which originates from micro crystalline graphite[176]. Figure 3-8 shows the Raman spectroscopy of both diamond nucleation side and growth side. The SEM image of the films used in this measurement has been plotted in Figure 3-5 (c) and (d). The detail
fabrication process of the devices will be shown in Chapter 6. From the plot, we can see that the ‘1332 cm\(^{-1}\)' peak shifts to 1305 cm\(^{-1}\) and two new peaks appear around 500 cm\(^{-1}\) and 1220 cm\(^{-1}\) for boron contents. The intensity of both new peaks of diamond nucleation side is lower than that of the diamond growth side, which indicates that diamond growth side has a higher boron doping concentration. Besides, a new broad peak for diamond nucleation side appears at around 1470 cm\(^{-1}\), which indicates impurity of graphite (sp\(^2\) carbon).

![Figure 3-8 Raman spectroscopy of BDD nucleation side and growth side. This experiment is done using the films and devices reported in Chapter 6.](image)

**3.4 BDD devices for chemical sensing**

Unlike the development of µLED light delivery strategies, where extensive efforts having been made, the engineering tools for *in vivo / in vitro* neurotransmitter detection is still under developed, especially for BDD electrochemical sensor. Most of the BDD neurotransmitter sensors fall into the category of BDD coated metal thin wires and BDD microelectrodes. Most of BDD coated metal thin wires share a similar topology as shown
in Figure 3-9(a) [177]. The diamond coated metal wire (Pt in this case) was attached to a longer copper wire using conductive Ag epoxy and inserted into a polypropylene pipet tip for insulation. Then the tip was carefully heated and softened, which allows polypropylene conformally flows over the BDD. Although those devices are convenient to fabricate, the capabilities of multi-site recording and integration with other functionalities are limited.

BDD microelectrodes utilize the emerging microfabrication technology and offer several advantages over BDD coated thin wires such as high density, high spatial resolutions, and easy mass production. However, most diamond growth substrates, such as silicon (with Young’s modules $\sim 10^{11}$ Pa[178]), are very rigid and do not match up with soft tissue (with Young’s modules $\sim 10^3$–$10^5$ Pa[179],[180]). Considering that diamond cannot grow on flexible polymer substrate due to high synthesis temperature, the flexibility of BDD microelectrodes is the barrier of applying such devices into implanted devices. To overcome this barrier, possibilities of making flexible BDD microelectrodes have been explored such as etching / thinning down diamond growth substrate and transferring BDD onto a foreign flexible substrate.

A thinned down diamond neural probe was developed by Chan et al.[181] as shown in Figure 3-9 (b). In this process, the undoped diamond was grown on silicon dioxide of a silicon wafer, on top of which, doped diamond (BDD) was grown and patterned as microelectrodes. The entire probe was released from silicon by etching down silicon wafer in hydrofluoric-nitric acid. In this case, BDD is sandwiched between top silicon dioxide passivation and bottom undoped diamond. While flexibility was shown due to the diamond substrate being extremely thin (3µm), a thicker substrate with greater lateral dimension is
desired to have stronger mechanical strength and application versatility. In viewing such drawback, a wafer transfer process was proposed by Hess et al.[182] and also reported by Bergonzo et al.[183], as shown in Figure 3-9 (c) and (d), respectively. In this process, silicon diode was used as a sacrificial layer and spin-cast epoxy, such as polyimide, was used as a flexible substrate. In detail, BDD was grown on silicon dioxide of a silicon wafer followed by patterning as microelectrodes and contact pads. Then polyimide was spun on and cured. The BDD devices were released from the silicon wafer by etching away silicon dioxide layer in hydrofluoric acid (HF). The thickness of these devices is determined mainly by the
polyimide, which can be controlled by spin speed. However, such process can only transfer small features such as electrodes and contact pads due to insufficient bonding strength between BDD and polyimide. Besides, it requires multiple steps of HF etching since the etching rate of silicon dioxide under larger patterns are faster than smaller patterns. In addition, the bonding strength between diamond and polymer is unknown. To the best of my knowledge, there are no published results on transferring wafer/large scale all diamond patterns from rigid diamond growth substrate on to a flexible substrate, which will be the focus of my work presented in Chapter 6.
4.1 Motivation

Based on the review on the light delivery methods of Optogenetics, this chapter proposed a method of making optical probes for Optogenetics-based deep brain optical stimulation using SU-8. SU-8 is a negative photoresist, which has been used in many biomedical applications. SU-8 has ~95% optical transmittance around 460nm wavelength, which is the maximum activation wavelength of the opsin used in this work. Therefore, by μLEDs at the tip of an SU-8 probe and directly inserting the light source into deep brain regions, attenuation caused by light transmission in wave-guided structures such as optical fibers or optrodes can be minimized. Besides, compared to silicon neural probes, SU-8 has a smaller Young’s modulus[185], [186], which makes it more flexible and thus causes less damage to the brain tissue. In addition, Additional Parylene-C

Figure 4-1 Conceptual diagram of the optical probe: (a) an overall view (b) a cross-section view.
encapsulation can potentially improve the long-term biocompatibility and reliability of the device for chronic implantation. In this chapter, design considerations of the SU-8 optical probe are described first, followed by the fabrication process. The optical, electrical properties of the as-fabricated device are presented. Finally, the functionality has been proven in vivo by clearly light-induced neural activity.

4.2 Optical probe device design fabrication

4.2.1 Device design

In the design, SU-8 2000 negative photoresist is used, which is an improved formulation of SU-8. It can achieve over 200 µm thick coating with a single spinning process. Besides, it is capable of making high aspect ratio structure and has very high optical transmission over 360 nm, which makes SU-8 2000 a perfect candidate for this design purpose [186]. As shown in the concept diagram (Figure 4-1), two layers of SU-8 photoresist were used in the fabrication, in which the top layer was 10% wider than the bottom one to encapsulate µLED chip. Several SU-8 anchors along the central line of the shank were designed to improve the bonding strength between the two SU-8 layers. The µLED chip end of the SU-8 shank is 10% smaller than the contact end to make it easier for penetrating into the brain. The overall device is encapsulated with Parylene coating to further protect the device from exposure to body fluid. The Parylene package also reduces the risk

Table 4-1 Dimensions of the SU-8 probe and µLED chips (L: length, W: width, H: height)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe (L×W)</td>
<td>4.2mm×0.86mm</td>
</tr>
<tr>
<td>Top SU-8 layer (W×H)</td>
<td>0.49mm×0.28mm</td>
</tr>
<tr>
<td>Bottom SU-8 layer (W×H)</td>
<td>0.42mm×0.12mm</td>
</tr>
<tr>
<td>Anchor diameter</td>
<td>0.03mm</td>
</tr>
<tr>
<td>Anchor interval</td>
<td>0.4mm</td>
</tr>
<tr>
<td>µLED (L×W×H)</td>
<td>0.550mm × 0.290mm × 0.100mm</td>
</tr>
</tbody>
</table>
of wiring being broken due to the dangling and stretching during device implantation. The dimensions of the probe and the µLED chip are listed in Table 4-1.

4.2.2 Fabrication process

The fabrication process is shown in Figure 4-2. In detail, a 3 inch silicon carrier wafer was cleaned by isopropanol, acetone and deionized water. (a) 5µm Parylene C was deposited (PDS 2010, Specialty Coating System, Inc), followed by spinning the first layer of ~120 µm SU-8 2075. (b) The first layer of SU-8 was patterned using standard lithography. (c) A 0.5 µm layer of Cu was deposited on the first SU-8 layer using a thermal evaporator (Auto 306, Edward, Inc). (d) The Cu layer was patterned and wet etched to form a connection between pads and leads. While copper was used in the prototype as a proof-of-concept, devices for chronic implantation will use a noble metal such as gold and platinum. During the copper patterning step, because the optical probe stuck out of the wafer, thick photoresist residue was observed at the edge of the probe after photolithography. As a result, a short circuit was formed between the anode and cathode of µLED, as shown in Figure 4-3(a). This is solved by increasing photoresist spinning speed by 50% and reducing 20% exposure time, which can significantly reduce the residue without undercutting too much copper. (e) S1813 photoresist was spun on top of the copper leads and exposed contact areas with µLED chips. The surface was then treated with O2 plasma (PX-250 plasma system, Nordson, Inc) with 500 mTorr process pressure and 100 W power to remove photoresist residue on top of the anode and cathode openings. Then low melting point (LMP) solder (melting point at ~62 ºC, 144 ALLOY Field’s Metal, Rotometals, Inc) was applied to the interconnects of the copper leads. (f) The wafer was immersed in the acid solvent and suspended µLED chips (Samsung, Inc) were self-
assembled onto the corresponding anode and cathode contacts. (g) S1813 was removed using acetone followed by IPA and DI water rinse. (h) A \( \sim 280 \) µm layer of SU-8 was spun and patterned to form the probe shape with a slanted tip for easy penetration of rat’s brain without removing the dura. A dual spin coating process was used in order to achieve a better uniformity of the SU-8
coating and prevent wrinkles appearing by thermal stress during the post baking of SU-8. This was done by spinning the first layer of ~120 µm SU-8 and then another layer of ~160 µm SU-8. (i) The LMP solder was applied onto pads in the solvent bath. Copper wires were bond to pads by melting LMP. Epoxy was applied to strengthen the bonding between pads and copper wires. Then SU-8 optical probes were released from the silicon wafer. Then, the whole device was encapsulated with ~10 µm conformal Parylene C coating. A fabricated optical probe with a µLED mounted on the tip is shown in Figure 4-3 (b).

4.3 Results and discussions

4.3.1 Electrical properties

The current-voltage curve of the µLED was characterized using a 4145B semiconductor parameter analyzer, (Hewlett Packard, Inc), as plotted in Figure 4-4. The forward threshold of this µLED is around 2.6 V. The typical voltages applied to µLED and their corresponding input powers re-listed in Table 4-2.

![Figure 4-4 I-V curve of the µLEDs](image-url)
Table 4-2 Typical parameters using for optical stimulation

<table>
<thead>
<tr>
<th>Applied voltage</th>
<th>Input current</th>
<th>Power consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 V</td>
<td>11.2mA</td>
<td>33.6mW</td>
</tr>
<tr>
<td>3.2 V</td>
<td>22.6mA</td>
<td>72.4mW</td>
</tr>
<tr>
<td>3.4 V</td>
<td>38.2mA</td>
<td>130mW</td>
</tr>
</tbody>
</table>

4.3.2 Optical properties

The spectrum of light intensity coming through a ~280 µm thick top layer of SU-8 is shown in Figure 4-5 (a). The peak intensity was located between 450 nm and 460 nm, which is within the maximum activation wavelength region [187]. To study the light attenuation due to the SU-8 coating, the out-coupling light intensities of probes was measured with a 280 µm SU-8 coating and without SU-8 coating, respectively. The data was recorded from a digital power meter (Model 815 Series, Newport, Inc) through an RHA 2000 evaluation board (Intan Technologies, Inc). As plotted in Figure 4-5 (b), the light intensities of the probes with the SU-8 coating are 23 % and 7.1 % higher than those without SU-8, when the applied voltages are 3.2 V and 3.4 V, respectively. This could be mainly attributed to the curvature of the top SU-8 layer formed due to the thermally-induced polymer reflow, which effectively collimates the light of the embedded µLED chip. The SEM image, (HitachiS-4700, Hitachi, Inc) and profilometry, (NanoMap-500LS, Nanomap, Inc) of the probe surface were taken as shown in Figure 4-5(c), where the clear curvature is shown. However, it is also observed that, when the applied voltage increased to 3.6 V, the intensity of SU-8 coated probe was slightly lower (7.6 %) than that of the uncoated probe. This inconsistency may be due to the overheating effect of the µLED at a high input voltage, which deteriorates SU-8 and results in the reduced optical transparency. Besides, the high noise level of measurement instrument might contribute to this as well. The variance of the data recorded from evaluation
board is quite noisy as shown from the error bars. The scattering property of µLED light was studied in gelatin to mimic the brain environment, where the µLED was driven with 2.74 V input voltage and 7 mW total power consumption as shown in Figure 3(d). The light propagation profile was plotted in Matlab® (Figure 4-5 (d)) where blue spectra (450 ~ 495 nm) were extracted. The 90% of the light intensity was within an elliptical-shaped scattering boundary (~ 10 mm in width and ~5mm in length), which enables optical excitation of a large population of neurons.

4.3.3 In-vivo LFP signal recordings

In vivo local field potential (LFP) upon optical stimulation was recorded in the unilateral visual cortex of an anesthetized, channelrhodopsin-2 (ChR2) expressed rat as shown in Figure 4-6 (a). The rat was anesthetized with a mixture of ketamine and xylazine according to its weight. After the rat was fully down, it was placed on a towel with heating pads underneath, which prevents
Figure 4-6 Demonstration of the efficacy of the deep brain optical stimulation using the fabricated probe in the rat’s brain: (a) the experiment setup and (b) – (d) the recorded LFP with the µLED input voltage of 3.0V, 3.2 V and 3.4V, respectively.
the rat from losing too much its body temperature during the surgery. Then the head of rat was stabilized in a stereotaxic apparatus through ear bars. After shaving hairs, 70 % alcohol and betadine were used to clean and disinfect the skin of the skull three times in turn. Then a rostral-caudal incision was made to expose the bony skull. Drilling was used to remove skullcap to prevent bleeding and minimize the damage to the cortical area. A tungsten probe was used to record LFP that was attached to a hydraulic microdrive to precisely control the penetration depth. The light-evoked signal was amplified and recorded using an RHA2000 amplifier and evaluation board (Intan Technologies). Significant changes in neural activity were recorded when the µLED was driven by 3.2 Vpeak pulses with a 100 ms pulse width. A clear light-induced neural activity was observed in time-domain LFP (1~100Hz) as shown in Figure 4-6(c), which demonstrates the efficacy of deep brain stimulation using the fabricated SU-8 optical probe. As a comparison, LFP oscillations excited with 3.0 Vpeak (Figure 4-6 (b)) and 3.4 Vpeak (Figure 4-6 (d)) pulses were recorded. The results show that the average amplitude of LFP oscillations increased from 400µV to 750µV when the input voltage of LED was changed from 3.0 Vpeak to 3.4 Vpeak. This is mainly due to the increased light intensity, which can activate more neurons in a larger range.

4.4 Conclusion

This chapter reports a method of making SU-8 optical probes for Optogenetics-based deep brain optical stimulation. The electrical and optical analyses show that the SU-8 layer not only remains the central irradiation wavelength of the µLED but also effectively enhance the light out-coupling intensity. Although the successfully demonstration of the efficacy of SU-8 probe, there is one disadvantage of using SU-8 to drive µLEDs due to the low thermal conductivity of SU-8. The thermal property of the SU-8 probes will be discussed compared with diamond probes in the next chapter.
Chapter 5. A hybrid neural interface Optrode with a polycrystalline diamond heat spreader for Optogenetics

5.1 Motivation

In this section, to address the localized Joule heating issue, attempts was made by mounting µLEDs on a polycrystalline diamond (PCD) heat spreader, which can dissipate electrically-induced heat rapidly and uniformly, and thus minimize tissue damage during optical stimulation[146]. Compared to polymeric materials, such as SU-8 that has a thermal conductivity of 0.3 Wm\(^{-1}\)K\(^{-1}\), PCD has a better thermal conductivity up to 1800 Wm\(^{-1}\)K\(^{-1}\) at 300K [73].

In the following sessions, device design and microfabrication method are first introduced, where two different devices are presented, including single-shank and dual-shank optrodes. In particular, the thermal properties of the single-shank PCD optrode are analyzed both experimentally and computationally and compared with probes made of other materials. Then, the optical and electrical properties of the optrodes are characterized. Finally, the functionality of the dual-shank optrode is evaluated by recording light-induced action potentials from primary visual cortex (V1) of a rat expressing channelrhodopsin-2 (ChR2).

A concept diagram of the heat spreading behavioral of SU-8 and PCD probes is shown on the bottom left in Figure 5-1, where the outstanding thermal conductivity of PCD over SU-8 inspired our proposed PCD-based optrodes on the bottom right. The optrodes are designed to have either single or dual probe shanks (a dual probe shank optrode is shown...
Figure 5-1 Concept diagram of the proposed neural interface optrode with a PCD heat spreader.

Table 5-1 Single/dual-shank probe dimensions

<table>
<thead>
<tr>
<th></th>
<th>Single-shank probe</th>
<th>Dual-shank probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shank Dimension</td>
<td>0.9mm×5mm</td>
<td>0.9mm×5mm (each)</td>
</tr>
<tr>
<td>Base Dimension</td>
<td>2.38mm×6.5mm</td>
<td>2.38mm×6.5mm</td>
</tr>
<tr>
<td># of Recording sites</td>
<td>3</td>
<td>4 (2 on each shank)</td>
</tr>
<tr>
<td>Recording site separation</td>
<td>1mm or 0.5mm</td>
<td>1mm</td>
</tr>
<tr>
<td>Shank separation</td>
<td>N.A.</td>
<td>1mm</td>
</tr>
<tr>
<td># of stimulating sites</td>
<td>1</td>
<td>2 (1 on each shank)</td>
</tr>
<tr>
<td>Recording electrode Dimension</td>
<td>90~100µm in diameter</td>
<td>90~100µm in diameter</td>
</tr>
</tbody>
</table>

here). Each shank contains a blue µLED chip on the tip for optical excitation of neural activity and two microelectrodes for the recording of light-evoked signals. For the first
generation of the device, commercially available \( \mu \)LED chips are utilized (Samsung, Inc), with dimensions of 0.55mm (length) \( \times \) 0.29mm (width) \( \times \) 0.1mm (thickness). The specifications of our design are listed in Table 1. The overall dimensions of the optrode are determined mainly by the resolutions of the photolithography masks and laser cutting. The probe is packaged with a Parylene C coating, which has been demonstrated to have the longevity of protecting microelectronics for chronic implantation[188].

5.2 Methodology

5.2.1 Device fabrication

The fabrication process of PCD probes is shown in Figure 5-2, (a) PCD was grown on a molybdenum substrate using a 2.45GHz microwave plasma assisted chemical vapor deposition (MW-PACVD) reactor with 2-3kW microwave power in a methane and hydrogen mixture atmosphere (4% CH4, 160-240Torr) and released by thermal stress during cooling from growth temperature to room temperature. Then diamond was cut into 10mm by 10mm size chips to avoid large nucleation on the growing side. The side attached to molybdenum (nucleation side) was used for fabrication. (b) The diamond substrate was cleaned by sonication in isopropanol (IPA) and deionized (DI) water for 30min each and then in nitric acid at 80ºC for 30min to create an oxygen-terminated diamond surface for metal deposition. (c) A 3nm layer of Ti and a 0.5\( \mu \)m layer of Cu were deposited using a thermal evaporator (Auto 306, Edward, Inc). Ti was used as an adhesion layer to improve the bonding strength between Cu and PCD. A photoresist mask was patterned using a mask aligner (ABM, Inc) for metal patterning. Then the Cu/Ti layer was wet etched using ferric chloride (to remove Cu) and buffered oxide etchant (to remove Ti) to form microelectrodes, contact pads, and interconnect wires. While Cu was used for prototyping, noble metal, such
Figure 5-2 Fabrication process for making the proposed PCD probe. (a) PCD growth on molybdenum (Mo). (b) PCD release from Mo substrate. (c) Metal deposition and patterning. (d)-(f) µLEDs assembly. (g)-(h) Parylene C coating and patterning. (i) Probe shaping.

as gold or platinum, will be used for chronically implantable devices. (d) Photoresist (S1813, Microchem) was spun on the substrate and selectively patterned to expose the µLED pad areas. Oxygen plasma (PX-250 plasma system, Nordson March, Inc) at a power of 100W and pressure of 0.5Torr was applied for 5 min to remove photoresist residue on the pad areas. (e) Low melting point (LMP) solder (62 ºC, 144 ALLOY Field’s Metal) was applied in an acid bath[189] and the die-form LEDs were self-assembled onto the contact pads wetted with LMP solder. (f) The substrate was rinsed with acetone, IPA, and then DI
water to remove the photoresist layer. (g) A ~5 µm Parylene C layer was deposited on top of the probe as an encapsulation using a chemical vapor deposition (CVD) system (PDS 2010, Specialty Coating System, Inc). (h) Parylene C was then patterned using oxygen plasma (RIE-1701, Nordson March, Inc) and a photoresist mask at the power of 300W and pressure of 0.25Torr, in order to open the recording sites and contact pads for electrical interconnects. (i) Finally, the probe was shaped using a Nd:YAG laser with a power of 8-14W (UltraShape 5xs, BettonvilleInc). To connect the probe to external powering and recording electronics, the pad areas were covered with the LMP solder, and flexible thin wires or Parylene ribbon cables were assembled to the probe. The Parylene ribbon cables were made out of patterned metal sandwiched between two layers of Parylene C with LMP solder applied at the contact pads and flipped-bonded onto the contact pads of the PCD probe. Finally, epoxy was applied to the contacts to strengthen the bonding between the pads and wires.

5.2.2 FEM Simulation of Device Thermal Properties

Finite element method (FEM) simulation was performed in COMSOL® Multiphysics 4.3 (COMSOL®, Inc.) with heat transfer in solids module. A time-domain solver was used to solve equation (5-1), where \( \rho \), \( C_p \), \( k \), and \( Q \) are density, heat capacity, thermal conductivity, and heat source (or sink), respectively.

\[
\rho C_p \frac{dT}{dt} - \nabla \cdot (k \nabla T) = Q
\]  

(5-1)

A single-shank PCD optrode was simulated and compared to an SU-8 based optrode fabricated using the method reported in [190]. The thicknesses of the PCD and SU-8 probe were measured at 0.24-0.3mm and 0.22mm, respectively and were modeled as 0.27mm for
the PCD probe and 0.22mm for the SU-8 probe in the simulation. The copper interconnects were modeled as high conductive layers with a thickness of 500nm, as shown in Figure. 5-3(a). Two 4.5cm copper wires (~0.2mm in diameter) attached to the contact pads were modeled as heat sinks of the system. From the simulation, the volumes of these 4.5cm long copper wires were sufficient to dissipate the heat generated by the μLED, given the fact that increasing the length of copper wires will not change much of the maximum temperature variation along the probe shank. The Parylene C coating (~5μm) was not modeled because it was much thinner than the SU-8 and PCD (~200μm). The parameters of the materials used in the simulation are listed in Table 2 [73], [191], [80], [192], [193],[194].

Table 5-2 Simulation Parameters in COMSOL®

<table>
<thead>
<tr>
<th>Material</th>
<th>Density (Kg/m³)</th>
<th>Heat Capacity J/(kg·K)</th>
<th>Thermal Conductivity W/(m·K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamond</td>
<td>3000</td>
<td>0.512</td>
<td>1800</td>
</tr>
<tr>
<td>SU-8</td>
<td>1236</td>
<td>1200</td>
<td>0.3</td>
</tr>
<tr>
<td>GaN</td>
<td>6100</td>
<td>500</td>
<td>130</td>
</tr>
<tr>
<td>Copper*</td>
<td>8700</td>
<td>385</td>
<td>400</td>
</tr>
</tbody>
</table>

*From material library in COMSOL®

Figure 5-3 (a) Simulation model in COMSOL®. (b) Mesh generated for FEM simulation.
The geometry of the model was discretized using tetrahedral mesh with a maximum element size of 1.73mm, minimum element size of 0.0743mm, and maximum element growth rate of 1.35, as shown in Figure 5-3(b). The initial temperature was set at 24°C for the SU-8 probe and 25°C for the PCD probe, which are the same as in the experiment. The boundary of the model was set to thermal insulation as the probes were coated with Parylene and epoxy at the base, which has relatively low thermal conductivity (Parylene C: ~0.084 (W/m·K) [195]). A heat source boundary of 18.6mW calculated from the experiment was applied at the interface between µLED and SU-8/PCD shanks with a 100ms pulse. It is of note that the above simulation does not include tissue, in order to compare with the experimental studies where the device temperature was monitored in air to resemble the worst scenario of the device application. In the brain tissue, the temperature variation of the implanted device is expected to be lower than that measured in air, as the high thermal capacity of brain tissue can counteract the temperature variation.

5.2.3 Device Characterization

Several experiments were conducted to fully characterize the thermal, electrical, and optical properties of the optrode. In particular, the thermal images of both the PCD and SU-8 devices were obtained in air using a high-resolution infrared camera (Delta Therm HS1570 and DT v2.19 software) with a LED applied voltage of 3.4V at 1Hz and 100mS pulse duration. The thermal images were processed using MATLAB® (R2011a, The MathWorks) and compared with the simulated results. The electrochemical impedances were measured in saline (0.9% NaCl solution) at room temperature using an embedded electrode-impedance-testing module in an Intan RHA 2000 evaluation board (Intan Tech. LLC). The light intensity of the µLED chip was measured using a digital power meter.
(Model 815 Series, Newport, Inc) and read through the RHA 2000 evaluation board. The light-scattering property of the optrode was also studied in a scattering media (20% gelatin). The blue light was extracted and plotted in a three-dimensional (3-D) format using MATLAB®.

5.2.3 In-vivo Animal Experiments

In-vivo acute experiments were conducted in V1 of a rat (Long-Evans) to demonstrate the functionality of the as-fabricated PCD probe. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University. Prior to the in-vivo testing, the rat was transfected with ChR2 to enable the functionality of light sensitivity upon blue light illumination. For viral transfection, the rat was anesthetized with ketamine and xylazine and then placed in a stereotaxic apparatus with a heat pad underneath to maintain its body temperature. A rostral-caudal incision was made in the skin to expose the skull. Two holes, positioned rostral-caudal, were made through the skull overlying each hemisphere of visual cortex. Each site was injected with 1.0µL AAV-hSyn-hCHR2(H134R)-mCherry solution (1011-1012 vector genome (vg/ml), UNC Vector Core), with an injection rate of 0.1µL/min, using a micro syringe (5µL, Model 75 RN SYR and 100µL, Neuros Adapter Kit, Hamilton, Inc). After each injection, the syringe was maintained in place for an additional 5mins to allow the viral vector to diffuse away from the injection site. Once the injections were completed, the cortical openings were plugged with Bonewax and then covered with Gelfoam, after which the overlying skin was sutured. Then the rat was given 5cc of sterile saline subcutaneously to prevent dehydration during recovery, as well as an injection of buprenorphine for pain relief. Post-injection, and before device implantation, the animal was housed in the animal care facility. A period of three
weeks was allowed for the animal to recovery fully from the injection surgery, and for cortical expression of the ChR2 gene.

The functionality of the PCD optrode for simultaneous optical stimulation and electrical recording was tested 3-4 weeks post-surgery. Following the same stereotaxic procedure as used for the viral vector injection, the rat was anesthetized and the skull opened over the region where the two injection sites. The PCD probe was inserted into V1 of the left hemisphere of the rat with the right hemisphere serving as a vector-injected control. During the in-vivo experiments, the µLED chips were driven by a continuous pulse train with various input voltages for optical stimulation. The light-evoked neural activity was recorded through the integrated microelectrodes (Channels 1-4) and amplified through an RHD 2132 32-channels headstage and RHD 2000 evaluation system.

5.3 Results and discussions

5.3.1 Fabricated devices

Figure 5-4(a) and (b) show the diamond growth side (rough side) and the nucleation side (smooth side) of the PCD substrate used in our fabrication. The diamond growth side of the PCD substrate has large diamond grain sizes up to 700µm and is not suitable for microfabrication. The nucleation side attached to the molybdenum during diamond growth has a relatively smoother surface and was used to construct microelectrodes, contacts, and interconnect wires. To analyze the surface roughness of the nucleation side, a 3-D quantitative atomic force microscope (AFM) image of a 5×5µm² area was obtained (AFM, Dimension 3100, Bruker Nano), as shown in Figure 5-4(c). The root-mean-square (RMS) surface roughness was measured to be ~ 201.98nm. Figure 5-4(d) shows a fabricated PCD
Figure 5-4 Diamond growth side (rough side) of a PCD substrate. (b) Diamond nucleation (smooth side) of the same PCD substrate. (c) An AFM imagine shows the surface morphology of the nucleation side of the PCD substrate. (d) Fabricated PCD and SU-8 probe for heat dissipation measurement. (e) A fabricated single-shank PCD probe with Parylene ribbon cable for interconnection. (f) Fabricated single/dual-shank probes. Inset shows the μLED, recording electrode, and metal interconnects.
probe and an SU-8 probe used for heat distribution measurement. Both probes had the same geometry, metal interconnects layout, and a similar average thickness (0.27mm for PCD and 0.22mm for SU-8). The average thickness of the PCD chip was tapered from around 0.15 to 0.45mm and laser thinned down to ~0.27mm. Figure 5-4(e) shows a fabricated single-shank probe with a Parylene ribbon cable attached to an interface with the recording board. Figure 5-4 (f) shows fabricated single and dual-shank probes with a close-up view of a µLED, recording electrodes, and metal interconnects. Approximately 20% scaling down of the width of the probe can be achieved in the future by cutting off the redundant PCD substrate.

5.3.2 Optical and Electrical Properties

The electrochemical impedances of the electrodes at 1 KHz (Channels 1-4 in Figure 5-8) in saline were 89.0, 20.6, 38.5, and 54.8 Kohm, respectively. Channel 1 was found to have high impedance due to incomplete Parylene removal and therefore was not used in the in vivo studies. The variation of the impedances among different electrodes is caused by the uneven surface morphology of the PCD, especially the one with large diamond grains of 100µm. This affects the leverage of the substrate during photoresist (PR) spinning and leads to uneven PR mask thickness and Parylene etching across the substrate. Hence, the Parylene coating on the microelectrodes of the left side (Ch1 and Ch4) of the probe is under-etched compared with Parylene on the right side (Ch2 and Ch3). The light spectrum of the µLED in Figure 5-5(a) shows that the peak intensity was located between 450 nm and 460 nm, which is within the maximum activation wavelength region [196]. The light intensity of the µLED chip is plotted in Figure 5-5(b) (blue), as a function of the applied
The light intensity with the applied voltage of 3.2V, 3.4V and 3.6V at 1Hz and 10% duty cycle corresponds to 0.79mW/mm², 1.16mW/mm², and 1.55mW/mm², respectively. The power consumption was calculated by multiplying the pulsating voltage and current during the onset of the LED and then normalized to 1 sec. These three voltages will be applied to the µLEDs used later in acute animal studies, where the minimum light...
Intensity required for activating a ChR2 transfected ion channel is reported to be ~1mW/mm$^2$ [197]. To estimate the power consumption of the optrode, the instantaneous input current of the µLED was obtained by measuring the voltage drop across a 10ohm resistor in series with the µLED while being driven by 1Hz, 10% duty cycle pulses at different voltages. The voltage across the µLED was measured directly and the power consumption was as plotted in Figure 5-5(b) (green). The corresponding normalized power consumptions were 3.47mW, 6.38mW, and 26.82mW at the applied voltage of 3.2V, 3.4V, and 3.6V with 1Hz and 100ms duration, respectively. Figure 5-5(c) shows the light scattering profile of the µLED when the optrode was inserted into gelatin. In this study, the µLED was driven by square pulses with 3.4V amplitude, 1 Hz frequency, and 10% duty cycle. Images were captured using a camera (EOS Rebel T2i, Canon) and analyzed using MATLAB® to extract blue light spectra (with a wavelength of 450-495nm), as shown in Figure 5-5 (d). An effective area of optical stimulation, where the majority of the optical energy was delivered, was estimated to be around ~2mm by 2.5mm.

5.3.3 Thermal Properties

Figure 5-6(a)-(d) show the measured and simulated heat distribution of the SU-8 and PCD optrodes, when the LED was powered by 3.4V, 1Hz, 100ms pulses. It can be seen that the SU-8 probe accumulated heat at the tip due to the poor thermal conductivity of SU-8, while the PCD probe dissipated heat throughout the entire shank in less than 0.5sec without creating localized hot spots on the probe. Similar phenomena were observed in FEM simulation. During the onset of LED stimulation, the maximum variations of the device temperature relative to the ambient temperature were measured at 3.3°C and 2.5°C from
the experimental data and 1°C and 0.5°C from the FEM simulation for SU-8 and PCD probe, respectively. Figure 5-7(a) and (b) show the measured cooling curves of the PCD (solid line) and SU-8 (dash line) optrodes, after activating the µLEDs for 60sec with different input voltages. The maximum temperature variations of an SU-8 probe were around 2.2°C, 4.2°C, and 10°C for the applied voltages of 3.0V, 3.2V, and 3.4V, respectively, whereas the PCD probe has maximum temperature variations of around 0.5°C, 0.7°C and 0.9 °C at the same applied voltages. Furthermore, it can be seen that the SU-8
probe required more than 2 seconds in order to return to the baseline temperature. Hence, sequential stimulation pulses come before the probe can be cooled down, which results in significant heat accumulated on the probe, especially when the probe is driven by fast pulse trains with high applied voltages. In contrast, the PCD probe has smaller temperature rises and faster cooling rates than the SU-8 probe, thereby effectively reducing the localized heat.
accumulation. This is demonstrated in the following study, where the LEDs were powered by continuous pulse trains. We monitored device temperature versus time after the LED was switched on, and defined the steady-state temperature as the temperature at $t=1\text{min}$ when the maximum temperature along the LED was no longer cranking up. Figure 5-7(c) shows the instantaneous changes in the tip temperatures and the steady-state temperature variations of the SU-8 and PCD optrodes for six repetitive heating on-off cycles. Close-up views of the data for PCD were plotted in Figure 5-7 (d) and (e). The maximum temperature variations of the SU-8 probe with input voltages of 3.0V, 3.2V, and 3.4V were 2.4°C, 4.0°C, and 5.5°C relative to environment temperature (~22.25 ºC) during the first duty cycle (100mS), continued to increase to 3.7°C, 6.8°C, and 8.7°C within the first 7sec, and then stabilized at 3.6°C, 7.3°C, and 11.8°C. On the contrary, the temperature variation of the PCD probe with input voltages of 3.0V, 3.2V, and 3.4V were 0.3°C, 0.4°C, and 0.5°C for during the first duty cycle, continued ramping up to 0.5°C, 0.6°C, and 0.9°C within the first 7sec and then stabilized at 0.5°C, 1.0°C, and 1.3°C. The maximum increase of transient temperature is around 1.0°C for the PCD optrode, which satisfies the safety requirement for implantable electronics[21]. The settled temperature variations for PCD probe were lower than the initial variations in all three applied voltages. For the SU8 probe, the settled temperature variation with an applied voltage of 3.0V and 3.2V were similar to the initial readings. However, the settled temperature variation with the applied voltage of 3.4V was nearly 3.8°C higher than the initial variation.
5.3.4 *In-vivo* Optical Neuromodulation and Recording

Figure 5-8 shows the experimental setup of the acute animal studies and the layout of a dual-shank optrode. During the optical stimulation, the µLED on the left side of the probe was driven by repetitive pulses with a frequency of 1Hz, the pulse width of 10ms, and amplitude of 2.9V, 3.2V, 3.4V, and 3.6V. Light-evoked neural responses were recorded from three functioning electrodes (Channels 2-4) and post-processed using MATLAB®. Figure 5-9(a) shows the neural signals recorded from Channel 2 with different applied voltages after applying a band-pass filter (60-3000Hz). Light-evoked action potentials were observed when the optical stimuli were switched from on to off with the high input voltage of 3.6 V, whereas no action potential was observed with 3.2 V and 3.4 V inputs. This is because the input voltages of 3.2 V and 3.4 V of the µLED may not be sufficient to evoke any action potentials, considering the tissue scattering and adsorption of LED light. Figure 5-9 (b) shows the action potentials recorded from different channels with 40 trails aligned and stacked when the applied voltage of LED was 3.6V, which demonstrated the consistency of the recording. A higher-level photocurrent artifact signals were observed at Ch4 because Ch4 is closer to the µLED than Ch2 and Ch3.

![Figure 5-8](image)

Figure 5-8 (a) *In-vivo* testing setup. (b) Probe schematic shows the location of 4 recording channels.
Figure 5-9 (a) Recorded signals with the applied voltage of 3.2V, 3.4V, and 3.6V, respectively. (b) Action potentials recorded from different channels with 40 trials stacking at the input voltage of 3.6 V.

5.4. Conclusion

In this chapter, a PCD-based, hybrid optoelectronic neural interfacing probe was designed, fabricated, and characterized, which is capable of optical stimulation and simultaneous recording of neural activity. The experimental and simulation results show that the application of the PCD heat spreader can keep the temperature variations of the probe around 1.0ºC during optical stimulation, which not only reduces the risk of thermally-induced tissue damage but also improves the accuracy of Optogenetic experiments by minimizing biological interferences due to thermal effects. In-vivo testing was performed in V1 of an Optogenetic rat, which demonstrated the
functionality of optical stimulating and electrical recording of action potentials of ChR2 transfected neurons. Light-evoked action potentials were observed when the LED was driven by 3.6V input, 10ms duration, 1Hz repetitive pulses. No action potentials were observed at 3.2V and 3.4V LED inputs. Studies verified that only 3.6V input can result in sufficient light intensity exceeding the minimum requirement (1mW/mm²) for opening opsin ion channels.
Chapter 6. Large-scale, all polycrystalline diamond structures transferred on flexible Parylene-C films for electrochemical sensing

6.1 Motivation

Despite its many advantages of diamond as demonstrated in Chapter 5, diamond is a rigid material with Young’s modules of ~1000 GPa[73], which is several orders of magnitude higher than that of the brain tissues (~10³ to 10⁵ Pa[74]). The micromotion-induced strain between rigid implants and surrounding soft tissues has been hypothesized to cause a harmful immune response and even irreversible tissue damage[75]. Recently, mechanically flexible, polymer-based neural implants have shown promises as the next generation of implanted devices[43],[76],[77],[78]. For those devices, electrodes and interconnecting traces made of noble metals were constructed on soft polymeric substrates with low Young’s moduli, such as polydimethylsiloxane (PDMS) (360-870 KPa[79]), polyimide (2.5 GPa[72]), SU-8 (SU-8 2000, 2.0 GPa[80]) and Parylene (2.8 GPa[81]). Consequently, the overall effective Young’s modulus can be significantly reduced to minimize the mechanical mismatches between rigid metal and soft tissues. Unfortunately, unlike noble metals, BDD cannot be fabricated directly on a polymer substrate due to its high synthesis temperature (500 – 900°C[82]) exceeding the glass transition temperature of polymers. To address this issue, a wafer transfer process is required to transfer diamond patterns from diamond growth substrates, such as silicon, onto flexible polymer substrates. Previously, Hess et al.[182] and Bergonzo et al.[183] reported a method for making diamond-on-polymer electrodes. In their approaches, the boron-doped polycrystalline diamond (BDD) was selectively grown and patterned on silicon dioxide (SiO₂) substrate, and then, transferred onto spin-casted polynorbornene and polyimide by removing the SiO₂ sacrificial layer in hydrofluoric acid (HF). Whereas such processes allow
transferring microscale BDD patterns such as electrodes, macroscale interconnects and contact pads must be fabricated from metal separately. Additionally, a multi-step HF releasing process is required since the release of polymers and large metal patterns are faster than the small BDD electrodes[182].

In this chapter, an innovative method is introduced of wafer-scale transfer of all diamond macro/micro patterns from a diamond growth silicon substrate onto a flexible Parylene-C substrate[198],[199]. Parylene-C is a micromachinable, transparent, flexible, FDA-approved and USP class VI biocompatible polymer, which has been widely used as an excellent structural and packaging material in neural implants [200],[52]. As illustrated in Figure 6-1, this approach involving three key steps: pre-transfer patterning, BDD-to-Parylene transfer, and post-transfer fabrication. During the pre-transfer process (Figure 6-1-I), SiO$_2$ was deposited onto a silicon wafer as a sacrificial layer followed by BDD synthesis and patterning. After slight over-etching of SiO$_2$ to create undercuts around the BDD patterns, Parylene-C was conformally coated to form mechanical anchors that can improve the bonding strength between BDD and Parylene. A mesh structure was employed on large patterns to introduce more anchors for further enhancement of the bonding force. For BDD transfer (Figure 6-1-II), two methods for substrate removal were explored and compared: potassium hydroxide (KOH) etching and deep reactive-ion etching (DRIE). For KOH etching, a special wafer holder including a Teflon jig, KOH resistant O-ring, and chromium-coated C-clamps was designed to expose only the backside of the silicon wafer through the opening of the upper lid, while protecting the front side to prevent the delamination between BDD and Parylene during etching. After BDD transfer, a post-transfer process (Figure 6-1-III) was employed where the free-standing BDD-polymer film was attached on a wafer followed
Figure 6-1. Illustration of the fabrication processes for I. pre-substrate transfer patterning; II. Substrate transfer; and III. Post-substrate transfer processing.

by subsequent micromachining processes for electrical components assembly, interconnects and wiring.

### 6.2 Methodology

Figure 6-1 shows a detailed process flow. In the pre-transfer step, (I-a) 1-µm-thick SiO₂ was deposited on a 3-inch Si wafer using plasma enhanced chemical vapor deposition (PECVD) (PlasmaLab 80plus®, Oxford Instruments). (I-b) Microcrystalline BDD film was synthesized using a custom-designed 2.45GHz microwave plasma assisted chemical vapor deposition reactor (MWPACVD) with a gas mixture of hydrogen-diborane and methane at a temperature of 700°C. (I-c) Aluminum layer was sputtered as a hard mask for diamond etching (Denton Desk Top Pro Sputtering System, Denton Vacuum, Inc.). (I-d) The
aluminum is patterned via photolithography (ABM, Inc.) and etched using an aluminum etchant (Type A, TRANSENE, Inc.). Then BDD was plasma etched in an electron cyclotron resonance reactive ion etcher (Lambda Technologies, Inc.) using SF$_6$/Ar/O$_2$ with microwave power of 400 W and a radio-frequency (RF) bias of 100 W (150 V). Afterward, the aluminum mask was stripped using the aluminum etchant. (I-e) SiO$_2$ was over-etched in buffered oxide etchant (BOE) to create undercuts for forming Parylene anchors. (I-f) The wafer was treated with the Silane A174 adhesion promoter (Sigma Aldrich, Inc.), followed by a conformal coating of ~15 µm Parylene-C (PDS 2010, Specialty Coating System, Inc.).

During the transfer step, Parylene coating on the backside of the Si wafer was first removed in O$_2$ plasma (PX-250 plasma system, Nordson March, Inc.). For wet etching of silicon (II-a1), the wafer was attached to the jig and etched in the 35% KOH at 70 °C for ~9 hrs. For plasma dry etching of silicon (II-a2), the frontside of the wafer was bonded onto a 4-inch carrier wafer using polyphenyl ether and the backside was completely etched with repetitive SF$_6$ and C$_4$F$_8$ cycles in a DRIE system (SPTS Pegasus 4, SPTS Technologies, Ltd). (II-b) The BDD-Parylene layer was released from the carrier wafer by soaking the wafer in acetone, followed by isopropanol alcohol (IPA) and deionized (DI) water rinses. Afterward, (II-c) sacrificial SiO$_2$ was etched completely in buffered oxide etchant (BOE).

During the post-transfer step, (III-a) the wafer-size BDD-Parylene film was bonded to a carrier wafer using photoresist. (III-b) Ti/Cu was evaporated (Auto 306, Edward, Inc.) and patterned (ABM, Inc.) to form contact pads onto the BDD film. (III-c) and (III-d) Low melting point (LMP) solder (62 °C, 144 ALLOY Field’s Metal) was applied onto the contact pads in an acidic solution. (III-e) Discrete active electronics, such as µLEDs, (Samsung, Inc) were self-assembled on the pads. (III-f) After releasing the device from the
carrier wafer, flexible wires were soldered onto the pads using LMP as interconnects to testing instruments. Epoxy was applied to strengthen the bonding between the wires and pads. Finally, another 5 µm Parylene-C was deposited to encapsulate the device with the electrodes being exposed for sensing.

As a proof-of-concept, two different BDD-polymer devices were designed as illustrated in Figure 6-2. In particular, a µLED probe was constructed in order to demonstrate the post-transfer fabrication step and to test the mechanical robustness of flexible BDD patterns during folding and bending. The µLED probe consisted of two electrodes of 60 µm in radius for both the anode and cathode. The wires were 2.4 mm long with various widths of 200 µm and 50µm, resulting in different wire resistances (Figure 6-2(a)). Figure 6-2(b) shows the BDD electrochemical sensors designed in a three-electrode configuration with: a reference electrode (RE), a working electrode (WE) and a counter electrode (CE). The overall design dimensions of the sensor were 4×4.7 mm². The effectively exposed BDD areas of WE, CE and RE were around 0.8 mm², 0.48 mm², and
0.48 mm², respectively, calculated by subtracting the areas covered with Parylene anchors. To create more Parylene anchors, microholes with dimensions of 30× 50 µm² and center to center separation of 50 µm were uniformly distributed on large BDD patterns, such as contact pads.

### 6.3 Results

#### 6.3.1 Characteristics of the BDD films

A scanning electron microscope (SEM) image (6610V, JEOL Inc.) in Figure 6-3(a) shows the surface morphology of the diamond nucleation side. The diamond nucleation side, which was originally in contact with the SiO₂/Si substrate, was exposed after the transfer process and served as the sensing surface of electrochemical sensors. It shows a smooth surface mainly due to the small crystal sizes of BDD. Figure 6-3(b) shows the Raman spectrum (532 nm Laser, HORIBA Scientific Inc.) taken from the nucleation side of BDD, where the characteristic diamond band at 1332 cm⁻¹ (Sp³ carbon peak) decreases and widens, two new boron bands appear at ~473 cm⁻¹ and ~1209 cm⁻¹ and Sp² carbon peak appears at 1470 cm⁻¹. The Raman features are similar to heavily doped BDD thin films with boron doping concentration estimated in the order of 10²⁰ cm⁻³[175]. Table 6-1 summarized the film properties including the average surface roughness of diamond nucleation side was 10.8 nm measured by an atomic force microscope (AFM) (D3100, VEECO Inc.). The thickness of the BDD thin film was around 2.7µm for µLED probes and 3.7µm for chemical sensors estimated based on the deposition rate of the BDD synthesis system. Comparing the two etching approaches, KOH etching results in weaker BDD-to-Parylene bond than DRIE etching, mainly due to interface damage and delamination during 9 hours soaking in corrosive KOH. While DRIE etching eliminates chemical attack on
Figure 6-3 (a) A SEM image shows the surface morphology of the BDD nucleation side. (b) A Raman spectrum shows both boron and diamond bands from the BDD nucleation side.

Table 6-1 Summary of BDD film Characteristics

<table>
<thead>
<tr>
<th>Doping level</th>
<th>Surface roughness</th>
<th>Film thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>~10^{20} cm^{-3}</td>
<td>10.8 nm</td>
<td>μLED probes: 2.7 μm, Chemical sensors: 3.7 μm</td>
</tr>
</tbody>
</table>

BDD-Parylene bond, the heat generated by aggressive plasma etching causes a deterioration in mechanical flexibility and clarity of Parylene on some of the devices.

6.3.2 Mechanical properties of the transferred BDD-Parylene structure

Figure 6-4 (a) and 6-4(b) show a custom-designed KOH etching kit before and after assembly, which includes a custom-made Teflon etching jig, a KOH resistant O-ring (Mykin, Inc) and chromium-coated C-clamps (Graham field, Inc). Figure 6-4(c) shows patterned BDD structures transferred on a 3-inch wafer-scale Parylene-C substrate, with a close-up view of contact pads and μLED electrodes. The SEM image in Figure 6-5(d) shows Parylene anchors formed around the openings of the BDD mesh over large BDD patterns. The flexibility of the BDD-Parylene structure was tested by wrapping the device on the tip of a 2.5mm-diameter micro punch with the μLED turned off (Figure 6-4(e)) and
Figure 6-4 The custom designed KOH etching kit (a) before and (b) after assembly. (c) BDD patterns on a wafer-scale flexible Parylene-C substrate after removing the Si substrate. Microscope images shows contact pads and μLED electrode probes with 200 μm and 50 μm trace width. (d) A SEM image shows the mesh structure of the contact pads, where Parylene anchors, Parylene substrate and BDD are highlighted in white, green, and blue, respectively. (e) and (f) Flexibility of a BDD μLED probe wrapped around the tip of a micro punch, with μLED off and on. (g) and (h) Scotch tape® testing before(g) and after (h) peeling a BDD-Parylene sensor off the tape, with the BDD side facing down.
Figure 6-4 (cont’d)

(g) BDD side facing down

(h) BDD side facing up

Figure 6-4 (cont’d) Scotch tape® testing before (g) and after (h) peeling a BDD-Parylene sensor off the tape, with the BDD side facing down.

on (Figure 6-4 (f)), demonstrating the integrity of the conductive BDD wires during bending. The enhanced adhesion between BDD and Parylene was demonstrated using Scotch tape® peeling test Figure 6-4(g) and (f). During the testing, a BDD-Parylene sensor was pressed onto a piece of Scotch tape® with the BDD side facing down and then peeled off slowly by fingers. Afterward, the sensor and the tape were inspected under a microscope. We did not observe BDD delamination or damage after five peelings, suggesting a strong mechanical adhesion between the BDD patterns and the Parylene-C substrate.

6.3.3 Characteristics of the BDD-Parylene electrochemical sensors

6.3.3.1 Potential window

Figure 6-5(a) shows a fabricated BDD-Parylene chemical sensor. The electrochemical properties of the sensors were characterized using cyclic voltammetry (CV) (CHI604, CH Instruments, Inc.). First, the potential window of an as-fabricated BDD chemical sensor was quantified in 1.0 M potassium chloride (KCl) solutions, and compared with a commercial standard gold (Au) electrode (CHI101, CH Instruments, Inc.), as shown Figure 6-5(b). During measurements, BDD or Au electrode was used as WE, commercially
available platinum (Pt) (CHI 102, CH Instruments, Inc.) and Ag/AgCl electrode (CHI 111, CH Instruments, Inc.) were used as CE and RE, respectively, with a scan rate of 0.1 V/s. The result shows that the BDD sensor exhibited featureless background current from -2.0 V to 1.5 V and a higher potential window of 3.5 V than the value of 2.2 V reported in [201] under the same test conditions. The Au electrode shows reduction current at 0.5V and oxidation current at a potential higher than 1.5 V, which defines a potential window of 1.0 V. The BDD electrode shows a much wider potential window than the Au electrode, therefore providing a bigger degree of characterizing analytes.

Figure 6-5 (a) A fabricated BDD-Parylene sensor with three electrodes. (b) Voltammogram of Au and BDD electrode in 1.0M KCL solution vs. Ag/AgCl at a scan rate of 0.1V/s (CE: Pt electrode, RE: Ag/AgCl). (c) Voltammograms of BDD electrodes vs. BDD at various scan rates (CE: BDD, RE: BDD). The voltammograms are offset for better visibility.

6.3.3.2 Double-layer capacitance

The double-layer capacitance (C_{dl}) resulting from an electrical double layer effect determines
the background current of a redox system using either a potential control or potential sweep method. A lower double-layer capacitance can reduce the charging current and lower the background noise, which contributes to a higher signal-to-noise ratio of chemical sensing. The $C_{dl}$ is determined by Eq. (6-1), where $i_{av}$ is the average current of the forward and reverse sweep of CV, $v$ is the scan rate of CV, and $A$ is the electrode area[202]

$$C_{dl} = \frac{i_{av}}{vA}$$  \hspace{1cm} (6-1)

To quantify the $C_{dl}$ of the BDD electrode, CV measurements were performed in 1.0 M KCl solution with various scan rates. All the three electrodes on the same BDD sensor were used in the measurement. Figure 6-5 (c) shows the voltammograms with scan rates of 0.1, 0.5, 1.0, 2.0 and 3.0 V/s. Figure 6-5 (d) plots the average current of forward and reverse sweeps at -0.9 V vs. BDD with different scan rates. A linear regression equation ($y=24x+23$) with an $R^2$ number of 0.979 is generated and the slope of this equation defines the $C_{dl}$, which is 24 $\mu$F/cm$^2$.

6.3.3.3 Chemical redox characteristics

For a full evaluation of the electrochemical characteristics of the as-fabricated BDD sensor, the sensors were tested with both outer and inner-sphere electron transfer processes by CV. For outer-sphere electron, the one electron transfer, Ru(NH$_3$)$_6^{2+/3+}$ redox couple was studied. During experiments, BDD was used as the WE, CE, and RE. Figure 6-6(a) shows the CV curves of the sensor with various concentrations of Ru(NH$_3$)$_6^{2+/3+}$ (262005-250 MG, Sigma-Aldrich) in 1.0 M KCl solution at a constant scan rate of 0.1 V/s. The peak potentials ($E_p$), half peak potentials ($E_{p/2}$) and peak currents ($i_p$) of oxidation process for different concentrations were extracted and summarized in Table 2. In addition, the iR drop in the electrochemical cell caused by solution resistance, contact resistance, BDD thin film resistance, etc. was measured, which were 0.94, 1.46, 1.69, and 2.65 mV for the Ru(NH$_3$)$_6^{2+/3+}$ concentration of 0.6, 0.8, 1, and 1.5 mM, respectively.
Those values are almost fifty times smaller compared with $E_p$ and $E_{p/2}$ and can be ignored in the data analysis. The peak current of oxidation process vs. different Ru(NH$_3$)$_6$$^{2+/3+}$ concentrations is plotted in Figure 6-6(b) and fitted to a linear regression curve ($y=228.8x-14.63$) with an $R^2$ value of 0.991, indicating that this oxidation peak is assigned to Ruhex. The values of $|E_p - E_{p/2}|$ were

![Figure 6-6 Voltammograms](image)

**Table 6-2 CV with different concentrations**

| Conc. (M) | $E_p$ (V) | $I_p$ (μA/mm$^2$) | $E_{p/2}$ (V) | $|E_p - E_{p/2}|$ (mV) |
|-----------|-----------|-------------------|---------------|--------------------------|
| 0.6       | -0.051    | 115.75            | -0.138        | 87                       |
| 0.8       | -0.044    | 180.75            | -0.130        | 86                       |
| 1.0       | -0.051    | 209.38            | -0.140        | 89                       |
| 1.5       | -0.055    | 328.00            | -0.144        | 89                       |
calculated as shown in Table 6-2, which were 87, 86, 89, and 89 mV for the concentration of 0.6, 0.8, 1, and 1.5 mM, respectively. The experimental values were larger than the theoretical value of 58.5 mV calculated from 58.5/n, where n is the number of electrons transferred in reaction [202]. This implies a quasi-irreversible/irreversible electron transfer process with a small standard rate constant (kθ), during which an activation overpotential η beyond the Nernstian equilibrium potential (Eeq) is required to drive the system to achieve the same exchange current level determined by a reversible redox system, which leads to a relative larger Ep. The voltammograms with different scan rates in 2.0 mM Ru(NH₃)₆²+/³⁺ solution with 1.0 M KCl supporting electrolyte are shown in Figure 6-6(c). The peak currents of oxidation process vs. the square root of the scan rates are plotted in Figure 6-6(d). A linear regression (y=0.42x+0.52) was observed with an R² value of 0.985, indicating that, although mesh structures were introduced at the electrodes, the mass transfer process of such a system is still controlled by semi-infinite linear diffusion of macroelectrodes rather than spherical diffusion of microelectrodes.

6.3.3.4 Dopamine sensing characteristics

For evaluation of the BDD sensors with an inner-sphere electron transfer system, DA was studied by CV and chronoamperometry based on the chemical reaction in Figure 6-7(a), where DA is oxidized to dopamine-o-quinone with two electrons transferred in the reaction[203]. Figure 6-7(b) shows the voltammograms of the BDD sensor vs. BDD at a scan rate of 1.0 V/s with various concentrations of DA (H8502-10G, Sigma-Aldrich) in 0.1 M, pH=7.4 phosphate-buffered saline (PBS) buffer solution. The inset of Figure 6-7(b) shows the voltammogram of 100 µM DA after subtracting the background charging current in the PBS solution, where an anodic peak potential (Ep) was observed at 1.0 V vs. BDD with a peak current of 0.66 µA/mm². The corresponding half-peak potential (Ep/2), which defines the potential at the half peak current, is 0.816 V. The iR drops
at $E_p$ and $E_{p/2}$ were measured and calculated using the aforementioned method, which were 2.56 mV and 1.28 mV, respectively. These values are two orders of magnitude smaller than the values of $E_p$ and $E_{p/2}$ and can be neglected. The calculated value of $|E_p - E_{p/2}|$ was 184 mV, much larger than the theoretical value of 28.25 mV [202], indicating an electrochemically irreversible/quasi-irreversible process. Chronoamperometry was used to study the ability of BDD sensors to detect low DA concentrations. In our study, a single step of 1.0 V vs. BDD was applied and current decayed exponentially with time was recorded. Fig. 6-7(c) shows chronoamperograms with 0.5µM to 100 µM DA in 0.1 M, pH=7.4 PBS buffer solution. With different DA concentrations, the background corrected currents, which are calculated by subtracting the current of background PBS
buffer solution from the steady-state currents of chronoamperograms, are plotted in Figure 6-7(d). A linear response of the BDD sensor to DA for the concentration of 0.5 µM to 100 µM is observed ($R^2 = 0.999$) with a detection limit of 0.5 µM and a sensitivity of 0.21 µA/cm²·µM

6.4 Conclusions

In summary, this section presented a novel fabrication process of transferring all diamond structures from a diamond growth substrate, silicon, onto a flexible Parylene C substrate, which enables the fabrication of all diamond on polymer devices without any metal traces in a wafer scale fabrication fashion. The unique design of Parylene anchors greatly enhanced the bonding strength between BDD and Parylene-C. The integrity of BDD thin films was demonstrated by bending a lit µLED BDD device on the tip of a micropunch. The comparative study against traditional gold electrodes highlighted the advantages of BDD-polymer devices. Detailed experimental investigation shows that the as-fabricated flexible BDD chemical sensor has a much wider potential window and smaller double layer capacitance compared with traditional gold electrodes, which gives wider freedom on detecting various chemicals. The electron transfer process and mass transfer process were studied using outer-sphere redox couple Ru(NH$_3$)$_6^{2+/3+}$ and the capability of sensing various concentrations of DA was demonstrated using chronoamperometry with the linear response from 0.5 µM to 100 µM ($R^2 = 0.999$). The fabrication methodology proposed in this paper can be easily adapted into the fabrication of larger-scale flexible BDD devices for large area or multiple targeted areas of interest. Future efforts include the development of multi-function flexible BDD arrays for both neural electrical and chemical signal recording in vivo in a rat brain model.
Chapter 7. Conclusion

Recent advances in Optogenetics provide a unique neuromodulation technique, allowing optical control of genetically targeted specific neurons that express light-sensitive opsin proteins with sub-millisecond temporal precision. Applying Optogenetics into studying DA release and uptake dynamics, researchers can have a more controlled manipulation of the dopaminergic system and have an unbiased study on DA related neurological diseases. However, the engineering tools which combines optical stimulation, neuroelectrical recording and neurochemical recording in a compact, low power fashion has not been reported. The ultimate goal of this thesis is to develop such tools from material and device perspective, and gradually bring them from laboratory to the market.

The motivation of this thesis started from observing joule heating issue from an SU8 based µLED probes. To address such issue, polycrystalline diamond has been introduced as a heat spreader for the first time for making µLED neural probes. Targeting at dissipating joule heating more efficiently, a PCD-based optoelectronic neural interfacing probe has been designed, fabricated and characterized, which is capable of optical stimulation and simultaneous recording of neural activity. The experimental and simulation results show that the application of the PCD heat spreader can keep the temperature variations of the probe around 1.3°C during optical stimulation. In-vivo testing was performed in V1 of an Optogenetic rat, which demonstrated the functionality of optical stimulating and electrical recording of action potentials of ChR2 transfected neurons.

Besides, the very high thermal conductivity, polycrystalline diamond has many unique features for chemical sensing, such as a large potential window, low background current and resistance to surface fouling, and long – term implantation, such as biocompatibility and chemical
inertness. The combination of those superior properties makes polycrystalline diamond a very promising candidate for a neural interface that has both functionalities of optical neuromodulation and neurotransmitter detection.

The motivation of designing a wafer transfer process raised from the rigidity of diamond thin film that the micro-motion between diamond and surrounding tissue can cause a harmful immune response and the process intolerance that cannot synthesis diamond onto flexible polymer substrate directly. The wafer transfer process introduced Parylene anchors that can increase the bonding adhesion between transferred diamond and Parylene substrate, which has been demonstrated by peeling test. To study the electrochemical properties of the transferred diamond-polymer electrodes, an outer sphere redox couple Ru(NH₃)₆⁺²⁺⁻²⁺ has been used to study electron transfer process. Then quantitative and qualitative studies of redox dopamine / dopamine-o-quinone have been performed to demonstrate the efficacy of neurotransmitter detection.

The future work will involve the integration of individual modules such as μLEDs, neurotransmitter detection, and neuroelectrical signal recording. The interference of a simultaneous recording neuroelectrical signal and neurotransmitter detection could be a challenge since the applied voltage from chemical sensing may interference the recorded signals. This issue requires careful design on the sensing circuitries and post signal processing. The initial success of applying diamond-polymer devices into neural interface application has shone some light on a new generation of neural interface strategy. It should be anticipated that diamond-Parylene technology will become an emerging technique in the field of implanted devices in the near future.
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