

MICRO-FABRICATED IMPLANTABLE HYBRID NEURAL INTERFACES FOR OPTOGENETICS

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ABSTRACT

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Neural interfaces are a direct communication pathway between nervous systems and external environment enabling promising clinical treatments for neurological disease and events such as spinal cord injury, stroke, and traumatic major amputations. Existing artificial neural interfaces mainly use electrical signals to evoke sensation in the central- and peripheral- nervous systems (CNS and PNS respectively), and these electrical systems have become a powerful tool for decades. However, its limitations demand improved technology. Recent developments in optogenetics have demonstrated the ability to target specific types of neurons with sub-millisecond temporal precision via direct optical stimulation of genetically modified neurons in the brain.

Current optogenetics-based stimulation interfaces mainly use laser- or light emitting diode (LED)-coupled optical fiber, micro-LEDs (μ -LEDs) array, and a laser beam focused through a microscope as their light sources. For experiments with freely behaving subjects, however, only limited light delivery methods are available. These systems' poor spatial resolution limits their functionality, and the tethered optical fiber greatly restricts subjects' natural behaviors.

To address these limitations, a series of hybrid neural interfaces based on a polymer-based flexible μ -LEDs array has been developed, and these arrays are designed to provide a unique solution to the current demand for multichannel, bi-directional neural interface devices.

Depending on interface location, there are three different neural interfaces: Opto- μ ECoG array for epidural stimulation, three dimension (3-D) waveguide array for deep cortical stimulation, and slanted 3-D waveguide array for multi cortical layers stimulation. The Opto- μ ECoG array contains a transparent micro- electrocorticogram (μ ECoG) electrode array with integrated μ -LED bare dies. To further improve the spatial and depth resolutions of the optical stimulation via the μ -LED, two types of 3-D multi-LED arrays were developed by coupling micro-scale optical waveguides with LED chips using a polymer-based microfabrication technology. Integration of individually addressable μ -LED chips with varying-length microneedles enables precise light delivery to target neurons in specific cortical layers.

To demonstrate the wireless capability of the arrays, this author and colleagues proposed a wireless-powered, multichannel optrodes array that is capable of simultaneous light stimulation and electrical neural recording.

Based on the development of hybrid neural interfaces, an optogenetics-based cortical visual prosthesis has been designed for treating all forms of blindness. With the hybrid neural interfaces, the core hypothesis that epidural optical stimulation of microbial opsin expressing neurons in the primary visual cortex (V1) can induce phosphine, artificial visual sensation, and perception, has been tested.

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*In dedication to my parents for making me be who I am, my wife for being the woman of my life,
and my son for being a greatest gift of my life*

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CHAPTER 1

A hybrid neural interface with integrated LEDs for optogenetics

Brain machine interfaces (BMIs) provide a direct communication pathway between the brain and external devices, and enable a promising therapy to restore motor control in severely disabled patients suffering from events such as spinal cord injury, stroke, and traumatic major amputations [1]. Current BMIs techniques primarily use visual feedback to drive prosthetic devices, and lack direct sensory feedback from artificial limbs into the brain [2]-[5]. With these single-modality interfaces, experimental and clinical results have demonstrated the ability of non-human primates and severely disabled patients to use brain activity alone to control a robotic arm to reach for and grasp objects [6], [7]. However, dramatic performance degradation, caused by a lack of proprioceptive and somatosensory feedback, has been observed in patients suffering from complete large-diameter-fiber neuropathy, who experienced difficulty executing sophisticated tasks (e.g. drinking from a cup) [8]. Therefore, a reliable and bi-directional neural interface is needed to close the BMIs control loop and improve recognition and control abilities of the prosthetic devices [9], [10]. To date, significant progress has been made in developing bi-directional neural interfaces with both recording and stimulation modalities for closed-loop BMIs.

Existing artificial neural interfaces mainly use electrical signals to evoke sensation in the central- and peripheral- nervous systems (CNS and PNS respectively). These electrical systems have become powerful tools in electrophysiology and clinical neuroscience to treat neurological events, injuries, and diseases. For example, applications include upper and lower limb prostheses

for spinal cord injury and stroke; bladder prostheses; cochlear and brain-stem auditory prostheses; retinal and cortical visual prostheses; cortical recording for cognitive control of assistive devices; vagus nerve stimulation (VNS) for epilepsy, depression, and tinnitus; and deep brain stimulation (DBS) for essential tremor, Parkinson's disease, epilepsy, dystonia, and depression [11]-[13].

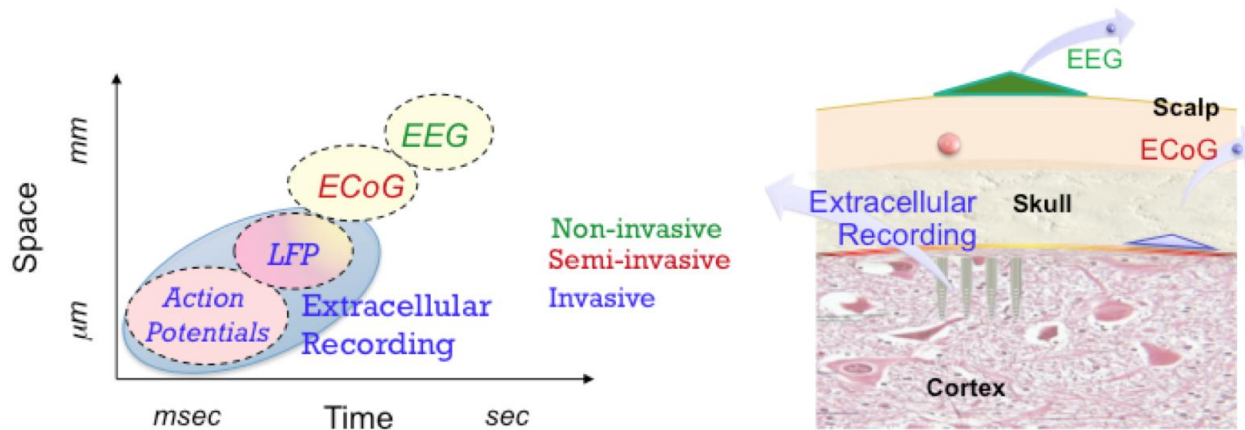


Figure 1-1. Types of neurophysiological signals and their temporal and spatial resolutions adapted from [14].

Depending on the location of the electrodes, the neurophysiological signal can be classified into three categories: electroencephalogram (EEG), electrocorticogram (ECoG), and extracellular recordings as shown in Fig. 1-1. Specifically, EEGs recorded from the scalp are the summation of the synchronous activity of large populations of cortical neurons. EEG recordings provide easy and non-invasive access to cortical neural activity, thus have been used extensively in various fields, such as neuroscience, cognitive science, clinical applications, and BMIs. However, the EEG recording suffers from poor spatial and temporal resolution caused by the filtering effect of the skull as well as artifacts from muscle movements [1].

Extracellular recordings—the observation of action potentials generated by a single or an ensemble of neurons and local field potential (LFP) from all nearby dendritic synaptic activity—can be obtained by direct implantation of voltage-sensing micro-scale electrodes. Action

potentials from a single neuron or a population of neurons have been used in many applications, and are considered the gold standard in electrophysiological studies [15]-[17]. Despite its many benefits, the extracellular recording requires invasive surgery to implant the recording electrodes, which surgery can result in complications and permanent tissue damage.

Similarly to EEG, ECoG also is the representation of synchronous cortical neural activity, but the neural activity is directly recorded from the cortical surface to circumvent the rapid signal attenuation effect of the skull [1]. ECoG has higher spatial resolution, broader bandwidth, and higher amplitude than does EEG [18]. Recently reported are micro-ECoG (μ ECoG) BMIs devices enabling a very high spatial resolution (360 channels in 2×3 cm) of neural recording [19]. Further, the ECoG is free of artifacts caused by muscle and eye movements consistently present in EEGs of awake and moving subjects. Finally, epidural ECoG recordings are less invasive than extracellular recordings, reducing risks of stroke, hemorrhage, and infection associated with penetrating electrodes, as well as signal instability in long-term use. A bi-directional ECoG interface also has been reported, showing that electrical pulses delivered through a μ ECoG electrode effectively can evoke sensation on the primary somatosensory cortex (S1) [20]. However, electrical stimulation through micro-scale electrodes often requires high current to activate neural activity, which can cause toxic redox reactions and damage to both the tissue and the electrodes. Moreover, the inherent limitations of electrical stimulation such as unpredictable current pathway, electrical artifact, non-specificity, and degradation of electrodes increase demands for a new technology [21].

Emerging optogenetics technology uses direct light stimulation to activate or inhibit genetically modified neurons. Optical stimulation provides many distinct advantages including

cell-type specificity, sub-millisecond temporal precision, rapid reversibility, elimination of electrical artifacts, and prolonged lifetime due to hermetic sealing of light sources [21], [22].

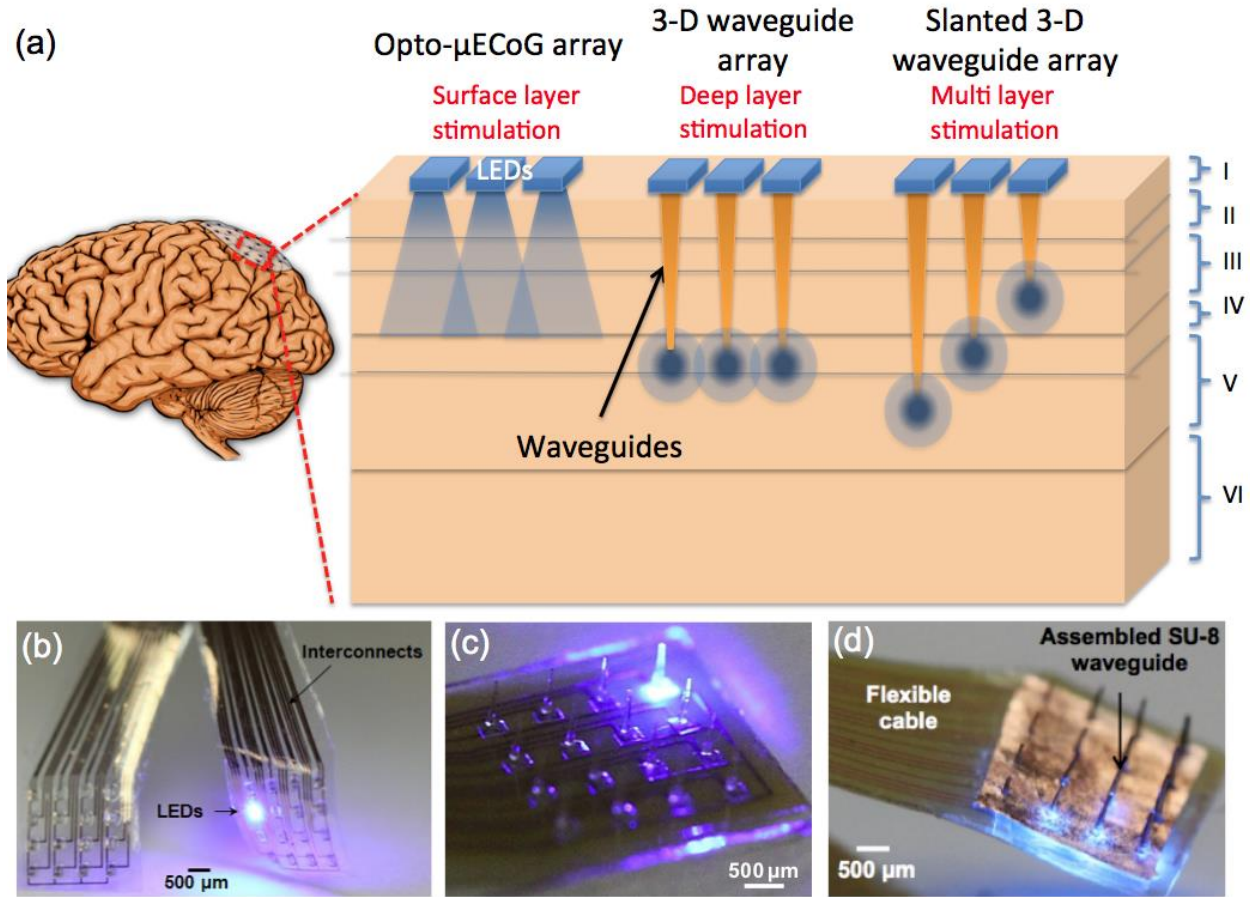


Figure 1-2. Different optogenetics-based hybrid neural interfaces for optical stimulation and electrical recordings of cortical neurons: (a) a conceptual illustration of the optical neuromodulator integrated with LEDs (b) Opto-μECoG array (c) 3-D multi-LED arrays coupled with micro optical waveguides (d) and slanted waveguides.

Current optogenetics-based stimulation interfaces mainly use laser- or light emitting diode (LED)-coupled optical fiber [23]-[25], micro-LED arrays [26], and a laser beam focused through a microscope [27] as their light sources. For experiments with freely behaving subjects, however, only limited light delivery methods such as a laser-coupled optical fiber and a head-mountable single LED system [28] are available. These systems' poor spatial resolution limits their functionality, and the tethered optical fiber greatly restricts subjects' natural behaviors.

To address these limitations, a series of hybrid neural interfaces based on a polymer-based flexible micro LEDs (μ -LEDs) array has been developed that would provide a unique solution to the current demand for multichannel, bi-directional neural interface devices.

Depending on interface location, there are three different neural interfaces: Opto- μ ECoG array for epidural stimulation, three dimension (3-D) waveguide array for deep cortical stimulation, and slanted 3-D waveguide array for multi cortical layers stimulation. The Opto- μ ECoG array contains a transparent μ ECoG electrode array with integrated bare chip μ -LEDs enabling optical stimulation and electrical recording. This author has demonstrated that a 32 individually addressable μ -LED array can evoke neural activities not only within the superficial cortical layer, but also within deep cortical layers ($\sim 600 \mu\text{m}$ deep) [29]. Although this 32 channel μ -LED array significantly improved spatial resolution of the optical stimulation, its depth resolution had a limitation.

A 3-D waveguide array integrating μ -LED chips with microneedle-shaped waveguides was developed to minimize scattering of LED lights in the brain tissue and achieve high spatial resolution in 3-D [30]. This author further improved the spatial resolution of the 3-D waveguide array in depth by a varying-length waveguide array [31]. Integration of individually addressable μ -LEDs with slanted microneedles allows precise light delivery to the target neurons in individual cortical layers. To create out-of-plane microneedles with various lengths, a polymer-based fabrication method was developed, utilizing the height variance in a dome-shaped, epoxy-based, negative photoresist (SU-8) structure. This method, dubbed a droplet backside exposure (DBE), independently can control the length, and tip/bottom diameters of individual microneedles without using a specialized machine or complex fabrication techniques. Fig. 1-2

shows a comparison of three different hybrid neural interfaces. Since these arrays are based on integrated light sources, they can be wirelessly powered and controlled.

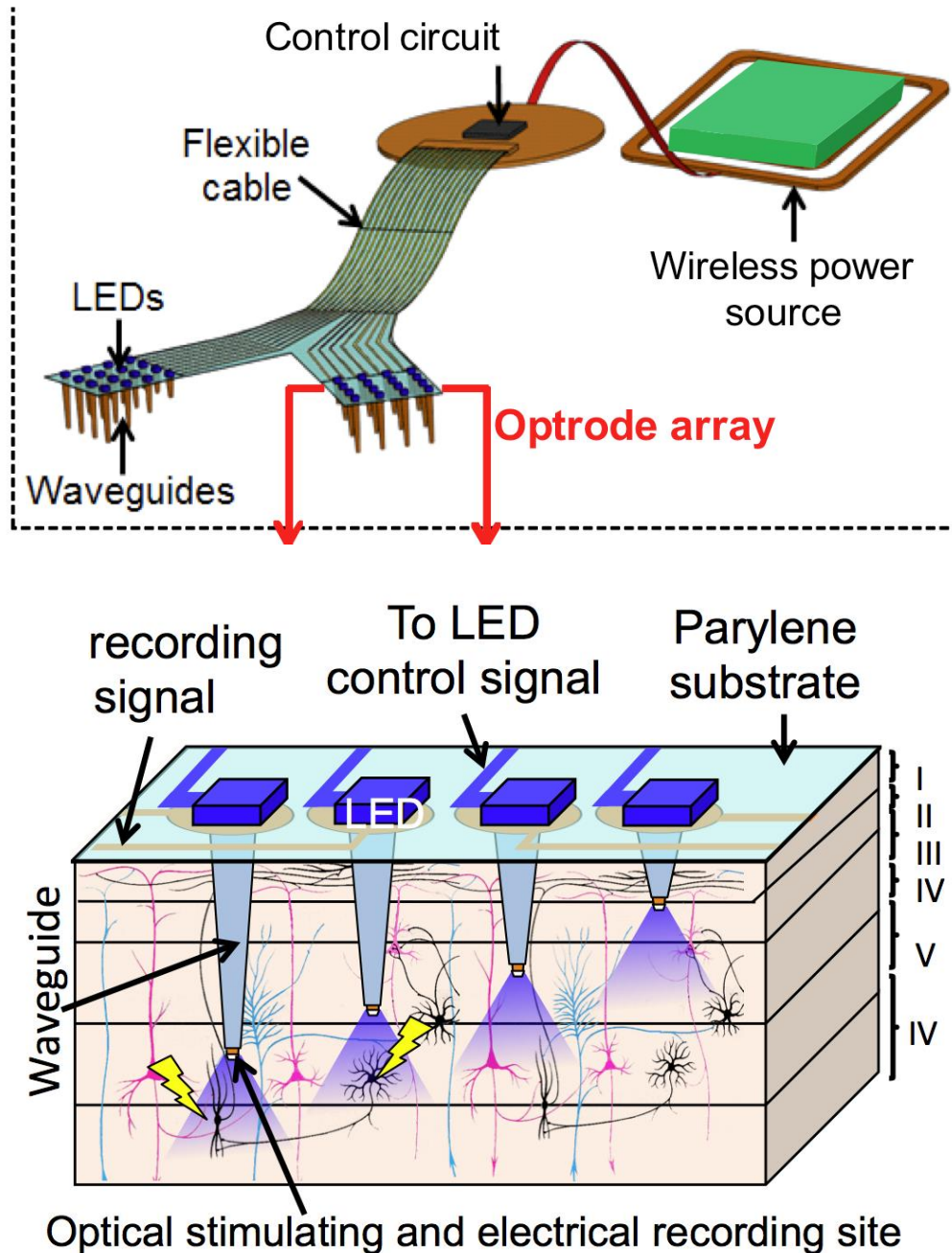


Figure 1-3. The overall design of the wireless optrode neural interface.

To demonstrate the wireless capability of the arrays, this author and colleagues proposed a wireless-powered, multichannel optrodes array that integrated μ -LEDs with microneedle

waveguides [32]. The waveguide arrays can be converted into optrodes, opto-electrodes for combined electrophysiological recording and optical stimulation array by utilizing metallic shielding layers as a recording electrode to achieve bi-directional hybrid neural interfaces. Recording electrodes wrapped around the waveguide cores permit simultaneous measurement of neural responses from various depths, upon optical excitation. Arrays were inductively powered and controlled by the wireless switched-capacitor stimulator (SCS), designed to improve power efficiency [33]. Fig. 1-3a and b depict the concept and design of the slanted optrodes array.

Based on the development of hybrid neural interfaces, this author is designing an optogenetics-based cortical visual prosthesis for treating all forms of blindness. With our hybrid neural interfaces, the core hypothesis that epidural optical stimulation of microbial opsin expressing neurons in the primary visual cortex (V1) can induce phosphine, artificial visual sensation, and perception, will be tested. In preliminary work, capability of the Opto-ECOG array for simultaneous optical stimulation and epidural recording of neural activities of the rat V1 has been demonstrated [34]. Several *in vivo* animal experiments have been conducted to study the correlation between neural activities in the V1 and light-evoked phosphenes including stimulus-driven neural data analysis, a chronic device development for freely behaving animal experiment, and a behavioral test in response to external stimuli.

This dissertation has organized the details of development of these neural interfaces and simulation and/or *in vivo* animal experiment results that have been demonstrated to evaluate the feasibility and functionality of the proposed hybrid interfaces for cortical visual prosthesis. The remainder of this report is structured as follows. In Chapter 2, background of optogenetics, available light delivery mechanisms, and specific examples about therapeutic treatment of neurological diseases based on optogenetics are reviewed. Chapter 3 presents design

specifications, device fabrication, and characterization of the Opto- μ ECoG array. *In vivo* experiments were performed to investigate the capability and reliability of the Opto- μ ECoG array. In Chapter 4, the design objectives, device fabrication and characterization of the 3-D waveguide arrays are discussed. A new fabrication process, called droplet backside exposure (DBE) method, for slanted microneedle structure, is presented in Chapter 5. Based on the devices and technologies discussed in previous chapters, a detailed plan for development of cortical visual prosthesis and current efforts in light-evoked neural data analysis are presented in Chapter 6. Chapter 7, as part of the development of cortical visual prosthesis, demonstrates chronically-implantable wireless flexible optrode array with multichannel μ -LEDs for selective optical stimulation of cortical neurons and simultaneous recording of light-evoked neural activity. Finally, in Chapter 8, the dissertation is closed via reference to a recent progress of the development of cortical visual prosthesis including the design of a behavioral animal experiment and battery-powered wireless neurostimulators for the experiment.

CHAPTER 2

A review of optogenetics and light delivery methods

2.1 Introduction

Optogenetics is a new neuronal modulation method allowing direct control of the activity of neurons by light. This can be achieved by genetically modifying the cell-types of interest to produce specific opsins, light-sensitive proteins. Once targeting cells express these opsins, they become light sensitive, and direct light exposure to the cells trigger neural activity [35]. Opsins are genetically encoded into the cells of interest, and this method allows targeting the specific cell population. Because each opsin has different characteristics, depending on the expressed opsins the cell can be stimulated or inhibited with light exposure of specific wavelengths [22]. Optical stimulation based on optogenetics is considered more beneficial than conventional electrical neuronal stimulation, because it permits activation or inhibition of specific types of neurons with sub-millisecond temporal precision, eliminates electrical artifacts, and potentially has a prolonged lifetime due to hermetic sealing of light sources.

This chapter reviews some recent advances in optogenetics and related technologies including the progress in development of new tools of optogenetics via genetic engineering, development of light delivery methods, and applications of optogenetics in the study and treatment of neurological diseases.

2.2 Light sensitive proteins

Optogenetics utilizes microbial opsin to modulate excitable cells. These opsins shown in Fig. 2-1 have been isolated from various microorganisms and plants, and channelrhodopsin (ChR) and

halorhodopsin (HR), naturally occurring photosensitive proteins, at first were used to induce activation or inhibition of neural activity in mammalian neurons [22], [36]. Since then, protein engineering has generated an expanded optogenetics toolbox for more precise and effective neuronal modulation including single-component proteins activated by various wavelengths of light, with various ion conductance regulation properties, enabling broad experimental configurations and opportunities [21], [37], [38]. Available single-component optogenetics tools can be categorized into four groups: excitation, inhibition, bi-stable modulation, and control of intracellular biochemical signaling in neurons and other cell types.

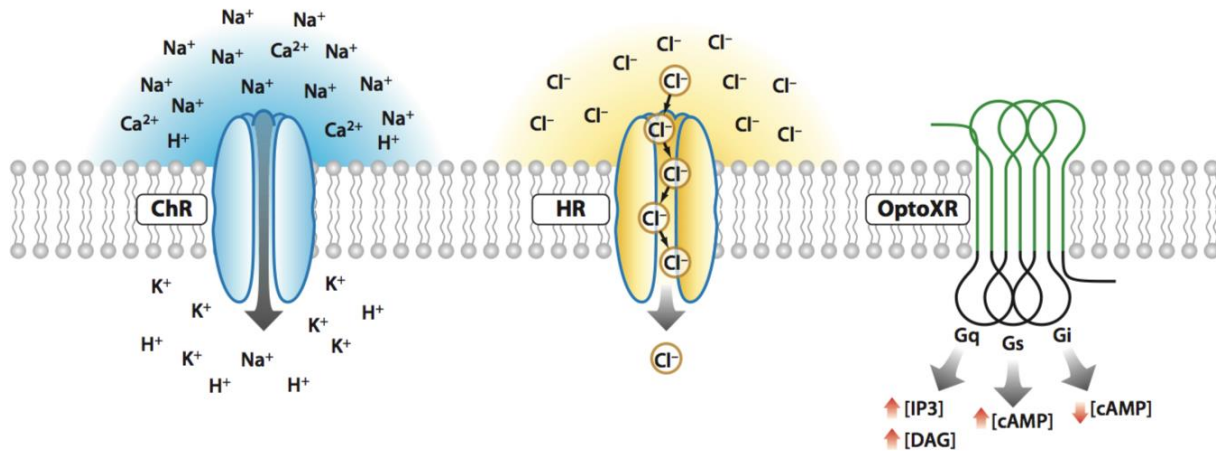


Figure 2-1 Basic properties of single-component optogenetics tool adapted from [39].

2.2.1 Excitation

ChRs are light-gated, non-specific cation channels in unicellular green algae that allow elective depolarization of genetically targeted cells [22], [40]-[45]. Molecular engineering of ChR has been very active over the last few years, and various ChR variants with distinct spectral and kinetic properties have been developed. Particularly, two mutations, H134R and T159C, achieved increased photocurrent amplitude by a factor of 1.5 to 2 in pyramidal cells [46], [47]. However, this improvement in photocurrent amplitude resulted in slower off-kinetics of 30 – 60

ms in neurons [48]. One of the key amino acids controlling the protonation state of the retinal chromophore is a glutamate at position 123 in the channelrhodopsin-2 (ChR2) sequence, and substitution of an alanine or threonine at this position generated E123A/T mutants, in which the photocycle is accelerated to an apparent off-kinetics of 4 – 6 ms [42]. These mutations can increase the reliability of high frequency evoked action potentials.

Table 1. Known single-component optogenetics tools for excitatory

Opsin	Mechanism	Peak Activation (nm)	Off Kinetics (ms)	
Blue/Green Excitatory				
ChR2	Cation channel	470 nm	10 ms	[36]
ChR2(H134R)	Cation channel	470 nm	18 ms	[45]
ChR2(T159C)	Cation channel	470 nm	26 ms	[40]
ChR2(L132C)	Cation channel	474 nm	16 ms	[41]
ChR2(E123A)	Cation channel	470 nm	4 ms	[42]
ChR2(E123T)	Cation channel	490 nm	4.4 ms	[42]
ChR2(E123T/T159C)	Cation channel	490 nm	8 ms	[40]
ChIEF	Cation channel	450 nm	10 ms	[43]
ChRGR	Cation channel	505 nm	4-5 ms	[44]
Yellow/Red Excitatory				
VChR1	Cation channel	545 nm	133 ms	[22]
C1V1	Cation channel	540 nm	156 ms	[35]
C1V1 CheETA (E162T)	Cation channel	530 nm	58 ms	[35]
C1V1 ChETA(E122T/ E162T)	Cation channel	535 nm	34 ms	[35]
C-VChR1	Cation channel	570 nm	85 ms	[52]
VCOMET	Cation channel	590 nm	100 ms	[44]
ReaChR	Cation channel	590 nm	137 ms	[53]

Initially available ChR variances were activated with limited light spectra that typically peak at 450 – 490 nm [43], [49]. These blue wavelengths have limited penetration depths into

neural tissue in mammalian systems, as they are strongly absorbed and scattered more strongly than yellow-red wavelengths [50]. The practical issue of efficient light delivery can be solved by creating a red-shifted ChR with spectral peaks near or above 600 nm, where light absorption and scattering by the tissue drop off steeply [35], [51]. An opsin from *Volvox carteri* (VChR1), which shares homology with ChR2 and works as a cation channel, can be activated by red-shifted light [51]. However, its relatively small currents due to low expression in mammalian neurons have limited *in vivo* adaptation. A new family of engineered chimeric opsin variants (C1V1) composed of channelrhodopsin-1 (ChR1) and VChR1 fragments implements fast and potent optical excitation at red-shifted (535 – 545 nm) wavelengths [52]. Red-activatable channelrhodopsin (ReaChR), a new variant of ChR, has improved membrane trafficking and expression in mammalian cells, a more robust spectral response above 600 nm and an enhanced steady-state response to light with wavelengths longer than 600 nm [53]. Since these ChR derivatives can be activated without affecting ChR2, there is a possibility of combinatorial excitation *in vivo* [52].

2.2.2 Inhibition

In principle, multiple different cell populations could be involved in the same circuit or behavioral property. For this reason, neural inhibition, in addition to these tools for neural excitation, can be used for studying the targeted cell population. NpHR is a light-activated chloride pump, hyperpolarizing the targeted neuron upon activation, with photocurrent peak at 590 nm and it can be found in multiple species of halobacteria [23]. Thus NpHR and ChR together create a powerful toolbox for neuroscientists by enabling multiple-color optical control for activating, silencing, and desynchronizing neural activity. Although optogenetics inhibition with NpHR was shown to operate well in freely-moving worms and in mammalian brain slices

as well as cultured neurons [23], [54], it suffered from membrane trafficking problems that must be addressed for use in behavioral studies in intact mammals.

Table 2. Known single-component optogenetics tools for inhibitory

Opsin	Mechanism	Peak Activation (nm)	Off Kinetics (ms)	
Yellow/Red Inhibitory				
eNpHR 3.0	Chloride pump	590 nm	4.2 ms	[56]
Green/Yellow Inhibitory				
Arch/ArchT	Proton pump	566 nm	9 ms	[57]
eBR	Proton pump	540 nm	19 ms	[56]
Red Inhibitory				
Jaws	Proton pump	632 nm	6.5 ms	[58]

Optimization of membrane targeting was required mainly for the inhibitory microbial rhodopsins, because most were derived from prokaryotic species in which membrane proteins are produced and transported using mechanisms significantly different from those in mammalian cells. This caused a tendency to accumulate in cells' Endoplasmic reticulum (ER). To improve proper assembly of these rhodopsins, Golgi and ER export motifs have been added to each end of the NpHR sequence [55]. That addition of the export motifs from mammalian ion channels reduced protein aggregation and increased the number of rhodopsin molecules reaching the plasma membrane [56]. Consequently, the most commonly used tools for neural silencing are the two enhanced versions: NpHR3.0 and Arch3.0 [37], [56], [57]. In a recent study, Chuang et al. introduced a red-shifted cruxhalorhodopsin, Jaws, for noninvasively mediating transcranial optical inhibition [58]. It is derived from *Haloarcula* (*Halobacterium*) *salinarum* (strain Shark) and engineered to result in red light-induced photocurrents three times those of earlier silencers.

Table 3. Known single-component optogenetics tools for bi-stable excitatory

Opsin	Mechanism	Peak Activation (nm)	Off Kinetics (ms)	
Bistable Modulation				
ChR2-step function opsin (SFOs)	Cation channel	470 nm activation / 590 nm deactivation	2 s (C128T) 42 s (C128A) 1.7 min (C128S) 6.9 min (D156A) 29 min (128S/156A)	[35] , [61]
VChR1-SFOs	Cation channel	560 nm activation / 390 nm deactivation	32 s (C123S) 5 min (123S/151A)	[35]

2.2.3 Bi-stable

Minimum activation light intensity for ChR2 mutations designed for faster off-kinetics was increased from 1 mW/mm² to 5 mW/mm², resulting in potential tissue over-heating [59]. To address this heating issue and to increase effective light sensitivity of optogenetic excitation, light-switchable variants have been engineered. Step-function opsins (SFOs) are a family of ChR mutants displaying bi-stable behavior: prolonged activity after termination of the light stimulus by bringing membrane potential close to the action-potential threshold and increasing the probability of spiking [60]. This can be achieved by mutations at positions C128 and D156. Single mutations C128S and D156A slow the apparent off-kinetics of ChR2 to 120 and 250 s, respectively [60], [61]. Combining both mutations generated a variant with a closing time constant of 30 min, and this stabilized SFO (SSFO) induces peak currents of >200 pA [52]. The SFOs also can be deactivated by a pulse of yellow light [60], hence these mutants can be activated by a short blue light pulse, then can be deactivated with a yellow light pulse allowing for precise control of network dynamics. Another advantage of SFOs is light sensitivity of the

expressed cells, due to photon integration during the entire duration of blue light illumination. This property renders the SFOs potential minimally invasive tools for stimulating large brain regions and deep brain structures.

2.2.4 Signaling

Table 4. Known single-component optogenetics tools for bio signaling

Opsin	Mechanism	Peak Activation (nm)	Off Kinetics (ms)	
Biochemical signaling				
Opto- β 2AR	G-protein signaling	500 nm	0.5 s	[62]
Opto- α 1AR	G-protein signaling	500 nm	3 s	[62]
Rh-CT(5-HT1A)	G-protein signaling	485 nm	3 s	[63]
bPAC	cAMP	453 nm	12 s	[64]
BlaC	cAMP	465 nm	16 s	[65]

Following the success of applying microbial rhodopsins as optogenetics tools in mammalian neurons, similar approaches have been applied to engineer a range of light-driven actuators allowing manipulation of diverse cellular processes [62]. One common approach involves addition or substitution of enzyme domains with photoreceptor units, which exhibit structural changes with optical stimulation [63]. In *Drosophila*, bPac, photo-sensitive adenylyl cyclase, expression increases the level of the signaling molecule cAMP by a factor of ten on blue light illumination [64]. Recently, the toolbox was extended by a light-inducible transcriptional effector for endogenous gene expression. A light-inducible dimerization of the cryptochrome Cry2 with its native truncated binding partner CIBN [65], was fused to a transcription effector

domain and a DNA recognition domain, respectively. Upon blue light illumination, the DNA bound CIBN recruits the Cry2-fused activator domain, and thereby can increase the transcription of target genes [66]. These and other tools have extended the range of optogenetics manipulation beyond the simple control of neuronal excitability and are likely to contribute significantly to a range of experimental applications [64].

2.3 Light delivery methods

Optogenetics has created a demand for optical devices targeting delivery of light not only on the surface but also to subregions of the brain. For surface light stimulation, several wide-field delivery systems have been proposed such as steering a focused laser beam using acousto-optic deflectors [67], digital micro-mirror devices (DMDs) [68], [69], and computer-generated holograms (CGHs) [70]. To deliver light inside the tissue, laser- or LED-coupled optical fibers are implanted in the brain [71]-[73]. Recently, more advanced methods based on microfabrication technology including channel waveguide arrays, arrayed optical fibers [74], [75], multiwaveguide fabrication on a single substrate [24], and polymer-based flexible micro-LEDs array [76] have been developed to increase spatial resolution of light in the three-dimensional structure of the brain. These methods can be categorized into three groups: waveguide-based systems, light distribution systems, and optrode-based systems, and details are discussed.

2.3.1 Waveguide-based systems

Since the most convenient and affordable lightguides for transmitting light over long distances are optical fibers, they are the most popular waveguide-based system for deep brain stimulation. In most applications, the beam of a solid-state laser or super luminescence LEDs is coupled to a multimode step index fiber, which guides and delivers the optical power to the region of interest.

Diode lasers and diode-pumped solid state (DPSS) lasers are in common use in optogenetics studies [22], [72], [77]. Main advantage of lasers is their highly collimated beam, which can be focused to a very small focal point. This is important for efficient coupling to thin optical fibers with core diameters of 50 microns or less. In addition, the narrow spectral width is helpful for selectively illuminating multiple opsins.

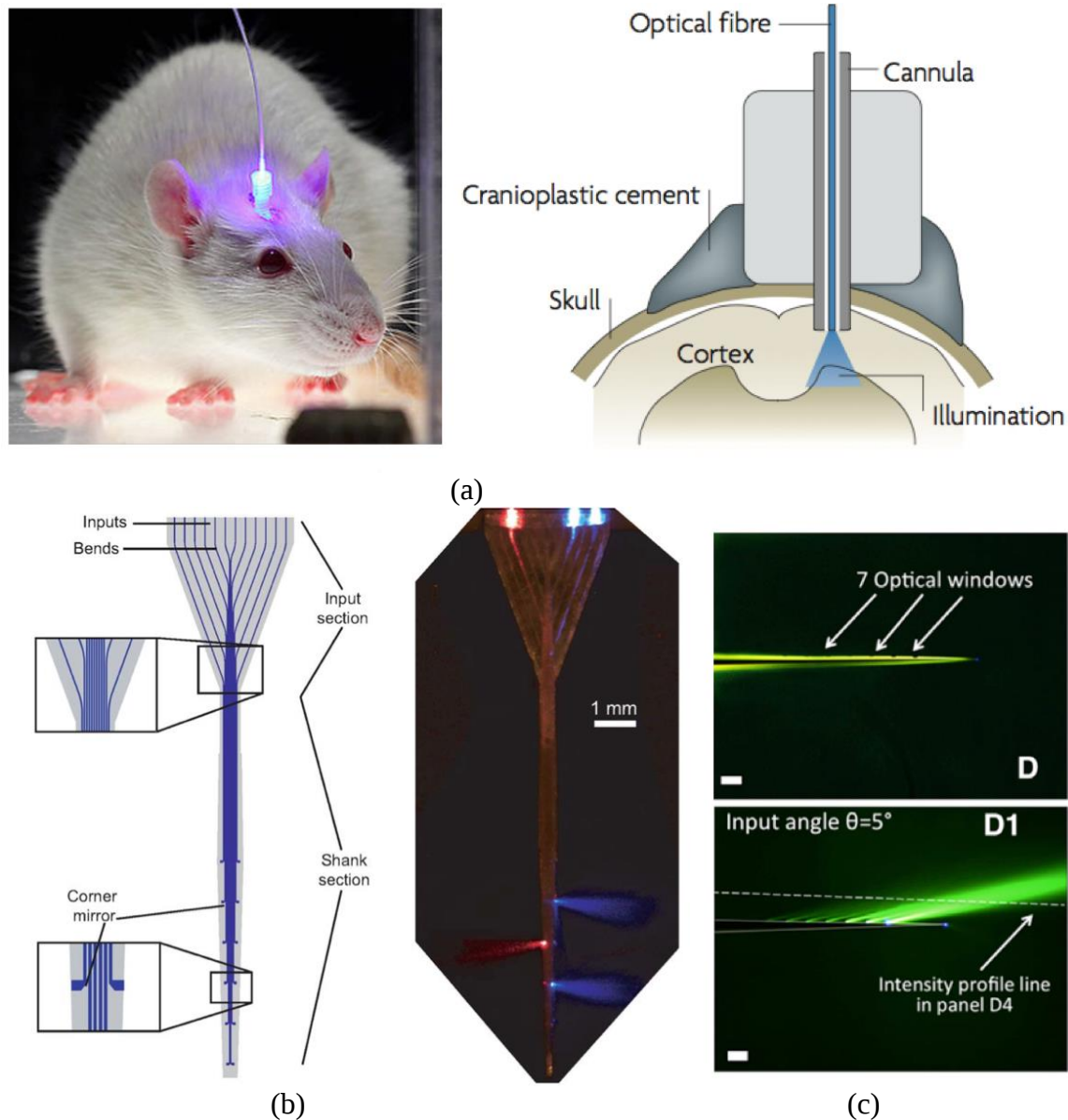


Figure 2-2. Waveguide-based light delivery methods: (a) laser- or LED-coupled optical fibers and the light delivery through an implanted cannula [22], (b) implantable three-dimensional sets of silicon oxynitride waveguides [79], and (c) multipoint-emitting optical fibers [81].

Using a LED illumination system for optogenetics studies provides many advantages including illumination stability and fast light switching [78]. Because LED systems do not require a resonant cavity like lasers, they do not have the noise related to emission modes or instability from back reflections that can be found with diode lasers. Precise temporal activation of LED systems is another advantage as a light source for optical stimulation, since optogenetics studies require well-defined temporal control. Most DPSS laser systems with TTL modulation input become unstable at fast switching rates, so mechanical fast shutters are necessary. LED can be directly modulated with TTL input for fast switching with a rise/fall time of microseconds. Electronically driven LEDs are particularly suitable for integrated systems such as prosthetic devices.

Optical fibers can be guided into the brain through an implanted cannula as shown in Fig. 2-2a [22]. In this method, viral infection and light delivery are in range, since the guide cannula is also used for virus injection. Light also can be delivered through implanted optical fibers [71]. In this case, a small piece of optical fiber is permanently implanted into the brain over the region of interest, and light is delivered into this fiber through an optical fiber connector mounted onto the animal's head. When compared with the cannulated light delivery, this approach has dual benefits: reducing the implant diameter, and coupling and de-coupling of the implanted optical fiber to a light source done easily with freely-behaving animals.

These methods are simple and reliable, but spatial control of light distribution is limited. It is possible to make an array of channel waveguides where each waveguide in the array terminates at a different depth. Recently, implantable three-dimensional sets of silicon oxynitride waveguides have been developed; raising the possibility of generating 3D distributed light patterns in the brain [79]. Individual waveguides can be addressed by a matrix of micro mirrors

or separately coupled to different light sources, allowing optical stimulation at each point with tunable wavelength and intensity (Fig. 2-2b). Another approach for 3D light distribution is physically to move an optical fiber inside the tissue [80]. In this design, a micro actuator moves a side-opened fiber, the optical fiber with an angled tip resulting orthogonal radiation pattern to the optical axis of the fiber, inside a glass cannula implanted in the brain prior to the experiment. This system can deliver different wavelengths at numerous positions inside the tissue. Recently multipoint-emitting optical fibers have been developed allowing selective and dynamic illumination of different brain regions along the taper (Fig. 2-2c) [81]. Site selection is achieved by a simple coupling strategy at the fiber input, and the use of a single tapered waveguide minimizes implant invasiveness. Light emission is permitted at selected sites along the taper by locally removing the coating to create “windows.” Illumination with a well-defined modal set at the fiber input then addresses emission to specific windows along the fiber [81].

While these methods allow spatially selective illumination, they require a complex fabrication process and/or coupling strategy at the distal end of the waveguides. Moreover, despite the wide range of proposed devices, only a few have been tested *in vivo* [75].

2.3.2 Light distribution systems

Distribution of light on the surface can be controlled by spatial light modulators (SLMs). Two types of SLMs are used to produce distributed patterns of light in the brain: digital micro-mirror devices (DMDs) based SLMs and liquid crystal display (LCD) SLMs. The DMD system is a light processor consisting of a large array of micro mirrors. When integrated into appropriate optical stimulation equipment, each mirror defines a pixel in a binary image that is projected onto the specimen. Individual mirrors can be switched independently with sub-millisecond precision [68], [82].

A LCD video projector has been modified to be used with lasers and coherent light sources [83]. In this design, a laser source produces a collimated beam of light, which is phase modulated by the LCDs and the modulated beam is launched into the optical path of a fluorescence microscope. The LCD SLM generates a dynamic mask, which reshapes a laser beam to control the distribution of light on the sample.

Steering a focused laser beam using acousto-optic deflectors (AOD) is another option for a surface-targeting light delivery method. Basic purpose of an AOD is to change rapidly the laser beam direction using the acousto-optic effect to diffract and shift the light frequency using sound waves, and it provides faster temporal resolution, typically on the order of a few microseconds, which is much faster than typical inertia-limited physically moving mirror-based scanning instruments [67]. Computer-generated holograms (CGHs) are optical devices enabling the projection of arbitrary patterns with high power efficiency [70], [84], [85].

2.3.3 Optrodes

An optrode is a novel dual-modality hybrid device consisting of optical fibers integrated with recording electrodes [86]. These devices are employed primarily for recording single-unit and multi-unit activity in anesthetized animals [74], [87], [88]. To introduce a fiber into a Utah-style multi electrode array (MEA), one of the electrode positions can be replaced with an opening (Fig. 2-3b) [86]. These devices often employ tapered fibers, which allow for reduction of the overall device dimensions but significantly increase optical losses and reduce the numerical aperture leading to reduced illuminated tissue volume. Silicon multi-electrode probes can be integrated with optical fibers by simply attaching the fibers on the electrode shank with an adhesive, following the fabrication process (Fig. 2-3 a) [74], [88]. These dual modal interfaces also can be extended by combining a microfluidic channel for fluid delivery [89]. A tungsten

microelectrode-based optrode that encloses optical fibers within its insulation glass was developed for targeting deep brain structure (Fig. 2-3d) [90]. Optical fibers and a tungsten wire were tightly bound to each other and integrally coated with a smooth, thin layer of glass. This design satisfied the structural requirements for use in deep brain structures.

These optrodes require an external light source, and a connector design remains one of the main challenges for simultaneous neural recording and optical stimulation, due to additional optical fiber coupling.

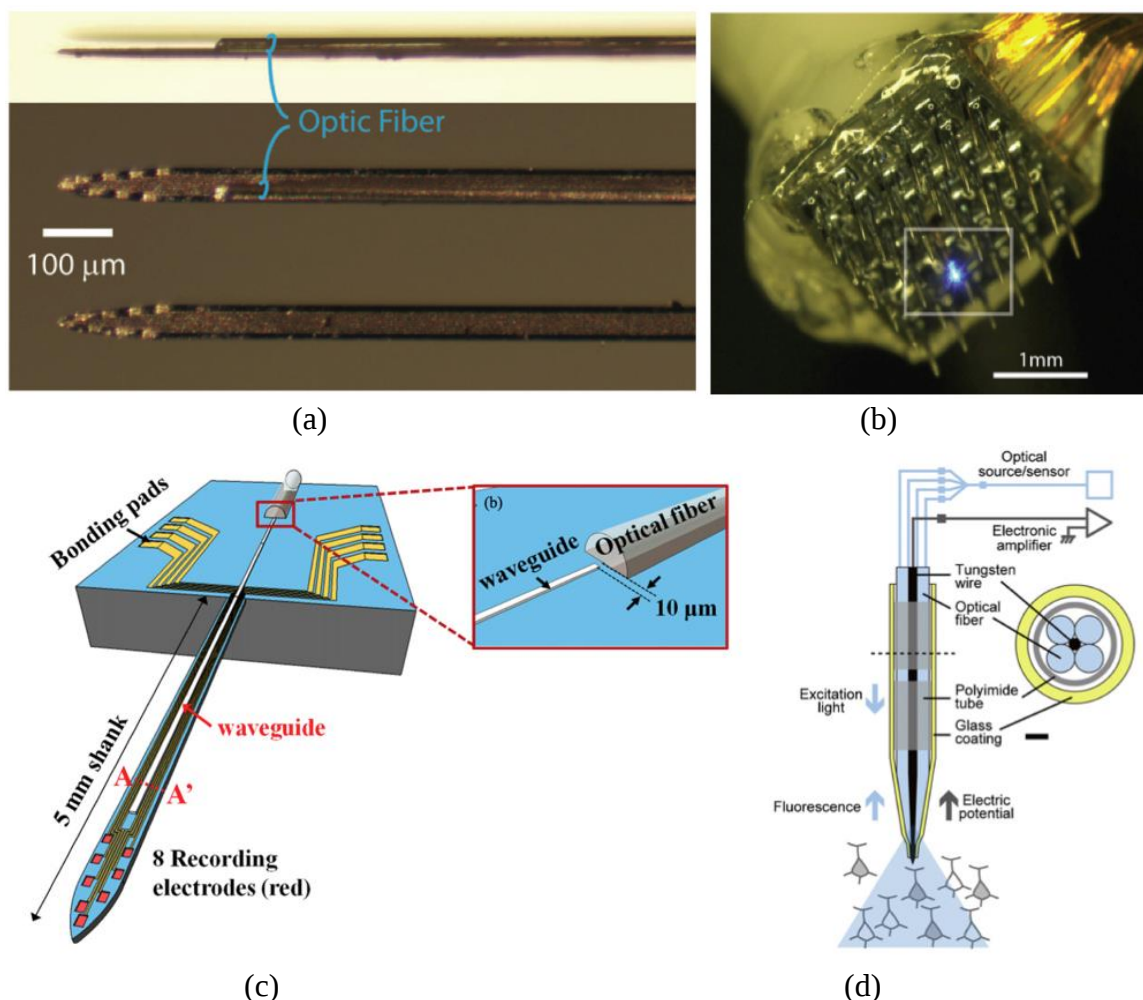


Figure 2-3. Optrode-based light delivery methods: (a) multi-array silicon probes with integrated optical fiber [74], (b) a Utah-style multi-array with an optrode [86], (c) a neural probe with monolithically integrated electrodes and waveguide [88], and a tungsten microelectrode-based optrode that encloses optical fibers within its insulation glass [90].

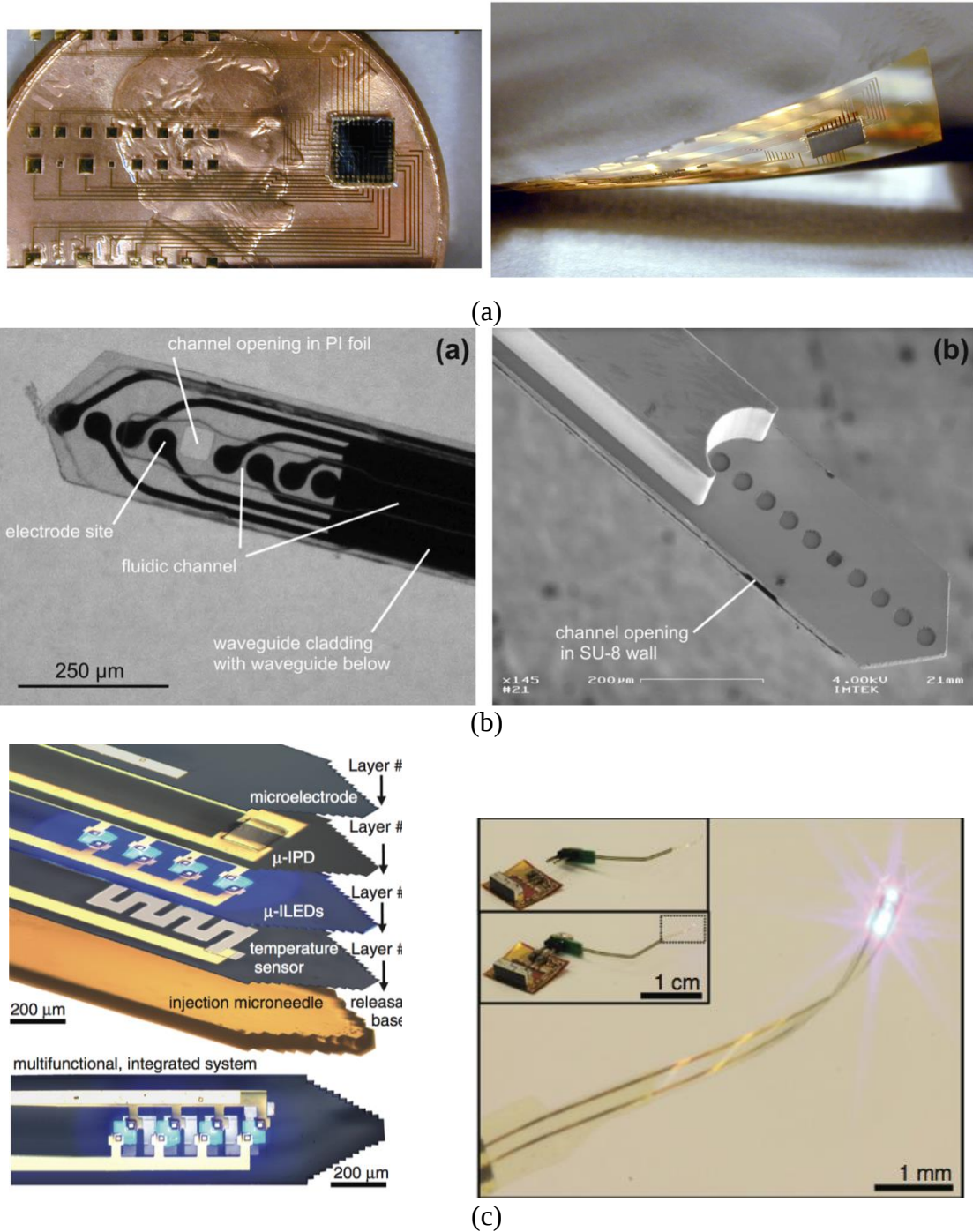


Figure 2-4. Polymer-based light delivery methods: (a) the chip level integration technology [96], (b) a polymer-based single optrode shaft integrated with microfluidic channel [89], and (c) a flexible optrode with integrated micro-LEDs using micro-contact printing process [99].

2.3.4 Polymer-based systems

Since the majority of optogenetics neural probes integrate rigid silica fibers, their mechanical

reliability would be compromised by optoelectronic integration. In addition, recent interest in the peripheral nervous system as a potential early therapeutic target for optogenetics demands more flexible and biocompatible platforms for the advancement of combined long-term optogenetics and electrophysiological studies.

Over the past several years, a number of technologies have developed to address these needs. Silicone resins (Polydimethylsiloxane (PDMS)) have been employed successfully as substrates for metal electrode arrays [91], [92]. This has allowed development of chronically implanted peripheral nerve cuffs capable of single-unit recordings from the nerve surface. Similarly, Parylene-C has been employed as a flexible substrate and as biocompatible packaging materials in various neural interfaces [93]-[97]. Li et al., developed an embedded chip technology to integrate commercially available IC chips with polymer substrates and passive components [95]. A placement cavity is etched on the Parylene-on-silicon substrate for chip placement. Once the chip is fixed inside the cavity, metal is deposited and a photolithography step is done to electrically connect pads on the chip to outside components (Fig 2-4a). The Parylene substrate to be integrated with the chip is a Parylene-metal-Parylene sandwich layer, that has been well characterized both *in vitro* and *in vivo* and is also approved by the Federal Drug Administration (FDA) for long-term human implantations. Several chronic and acute devices tested with histology results have been published to verify overall biocompatibility and feasibility of the sandwich layer technology.

The micro-contact printing process developed by Rogers and colleagues has been employed recently in fabrication of sophisticated integrated structures on highly flexible silicone, polyimide, and silk fibroin substrates [76], [98]. This approach takes advantage of the mature chip-scale semiconductor processing and combines it with patterned interconnects which enable

release and transfer of devices fabricated on wafers onto flexible substrates. In a recent study, a flexible optrode, shown in Fig 2-4c, was thus fabricated, integrating a single recording channel with LEDs, photodetectors, and a thermal sensor [99].

2.4 Specific studies based on optogenetics and their methods

Traditionally, neurological and psychiatric diseases of the brain were considered as loss or disruption of function due to changes in cell mass (neurodegenerative diseases), cell function (memory loss), neurotransmitter systems (Parkinson's disease), or perturbed connections (different types of addiction) [100]. Optogenetics has been used to identify clinically-relevant therapeutic targets to understand normal and disrupted function of neuronal circuits by assessing whether the activation or silencing of certain neurons treats certain disease states in animal models. Using microbial opsins and devices optimized for delivery of light into the brain as described above, researchers can monitor how specific cell types, projection pathways, and regions contribute to Parkinson's disease, epilepsy, and other human brain disorders in rodent models [101], [102].

Optogenetics-based circuit mapping has been initiated to understand local connectivity amongst various neuronal cell types [103], [104] and long-range connectivity between brain regions [105]. Activation and inhibition of these connections locally and at a distance may alter neuromodulation balance.

Understanding cortical circuits requires mapping connections between specific populations of neurons and dendritic locations where synapses occur. ChRs have been used to explore both synaptic potentiation and depression [103], [106], providing deeper understanding of circuit development and pathologies. Specifically, ChR2-assisted method [105] was developed to map spatial distribution of synaptic inputs, defined by presynaptic ChR2 expression, within the

dendritic arborizations of recorded neurons. This study demonstrated high specificity in the subcellular organization of excitatory circuits in a mouse model.

Distribution of local neuronal connections can affect regional inhibition *versus* excitation, and the SSFO was used to study a mechanism of different observations of changes in cortical excitatory/inhibitory balance [107]. This phenomenon might be linked to physiological states such as relative loss or hypofunction of medial prefrontal cortex (mPFC) inhibitory interneurons, altered gamma oscillations, and social and cognitive dysfunction in diseases such as autism and schizophrenia [107]. Using targeted expression of the SSFO in both excitatory and inhibitory cells of mPFC, a region that exerts significant top-down control over decision-making, impulse control, and many social behaviors, the balance of excitation and inhibition in this region could be altered selectively [35], [108], [109]. These studies have provided support for interneurons' role in the generation of gamma and in sharpening cortical sensory transmission and enhancing mutual information in connected cells. These results imply that loss or aberrant expression of inhibitory cortical cells, often observed in psychiatric disease, may lead to cognitive derangements seen in schizophrenia and autism [38].

A combination of circuit manipulations using optogenetics and behavioral phenotypes has been used to determine projection-specific targets within normal function and disease. Other conventional interventions such as pharmacological, transcranial magnetic stimulation, and electroconvulsive therapy were unsuccessful, partially due to lack of specificity of the treatment and understanding of the appropriate target circuit sites for intervention [100]. mPFC not only involves previously-mentioned social dysfunctional disorders, but also sends multiple projections to subcortical nuclei associated with depression. Warden et al. optically defined two heterogeneous circuits alternately leading to impulse control disorders and amotivational states,

initiated in mPFC [110].

Combining optogenetics with behavioral read-out methods was used to study effectors of nuclei within a circuit, and the relationship between patterns of activity within these nuclei and behavioral phenotype. For example, sleep disorders (e.g., narcolepsy) and consciousness (e.g., delayed emergence from anesthesia, coma) arise from derangements in pathways of the arousal circuit, a series of interconnected subcortical nuclei, and Adamantidis et al. demonstrated the mechanism using the combination of optogenetics with behavioral read-out method [77]. Direct stimulation of hypocretin-producing neurons within ChR2 expressed lateral hypothalamus (LH) increased the probability of sleep-to-wake transitions, and shortened the latency to wakefulness in a rate-dependent fashion. A similar causal role for norepinephrine in arousal was demonstrated by using a Cre-driver line to express ChR2 in the locus coeruleus (LC) [111]. These studies enable new targets for the treatment of narcolepsy, other disorders of sleep and arousal, and delayed emergence from general anesthesia.

The optogenetics approach also can be combined with functional magnetic resonance imaging (fMRI) [112]. Optogenetics functional magnetic resonance imaging (ofMRI) [112]-[115] technology combines optogenetics control with high-field fMRI readout enabling cellular-level precision in control while whole brain network function can be read out *in vivo*. While standard fMRI provided important clues to, and insights into, brain function including mapping of visual, sensory, motor areas, and association of brain activation changes relevant to neurological diseases, they were limited in accuracy due to the brain's densely interconnected network of heterogeneous cellular populations. ofMRI addresses this problem by combining genetically-targeted control of neurons with a method capable of whole brain functional readout.

CHAPTER 3

Opto- μ ECoG array

3.1 Motivation

Based on the previous chapter's review of light delivery methods for optogenetics, this author has proposed a hybrid opto-electrical system combining ECoG electrical recording and optical stimulation methodology. This polymer-based flexible micro neural interface would provide a unique solution to the current demand for multichannel, bi-directional neural interface devices. Whereas some studies have been reported, combining ECoG and optogenetics for cortical mapping [116] and neural modulation [117], several design challenges limit the development of a complete BMIs system for a chronic implant. Specifically, current optogenetics-based ECoG interfaces mainly use laser- or light emitting diode (LED)-coupled optical fiber [118] and SLM based systems [100] as their light sources, none of which suit BMIs in freely behaving subjects. In addition, densely arranged ECoG electrodes, made of opaque metals (e.g. platinum), cover a large portion of the cortical area, so optical stimulation of the cortical area through these opaque electrodes remains problematic.

To address these challenges, a novel Opto- μ ECoG array consisting of a transparent epidural micro-scale electrodes array for electrical neural recording and a LED array for optical neural stimulation was proposed (Fig. 3-1). The high-density μ ECoG electrodes array captures neural activity of large populations with relatively high temporal and spatial resolution. μ -LEDs, self-assembled on a flexible polymer substrate, enable optical modulation of specific types of neurons with sub-millisecond temporal precision, and allow the possibility of achieving a truly

untethered system crucial for chronic behavioral experiments. Parylene-C serves as the main structural and packaging material, because its properties - flexibility, optical transparency, chemical inertness, and biocompatibility - are favorable for implantable devices. In addition, epidural placement of the device on the surface of the cortex minimizes risk of brain tissue damage. In this chapter, design specifications of the proposed Opto- μ ECoG array are described, followed by device fabrication. Next, the optical, electrical, and thermal characteristics of the fabricated device are presented. Finally, the *in vivo* experiments that were performed to investigate the capability and reliability of the device are explained.

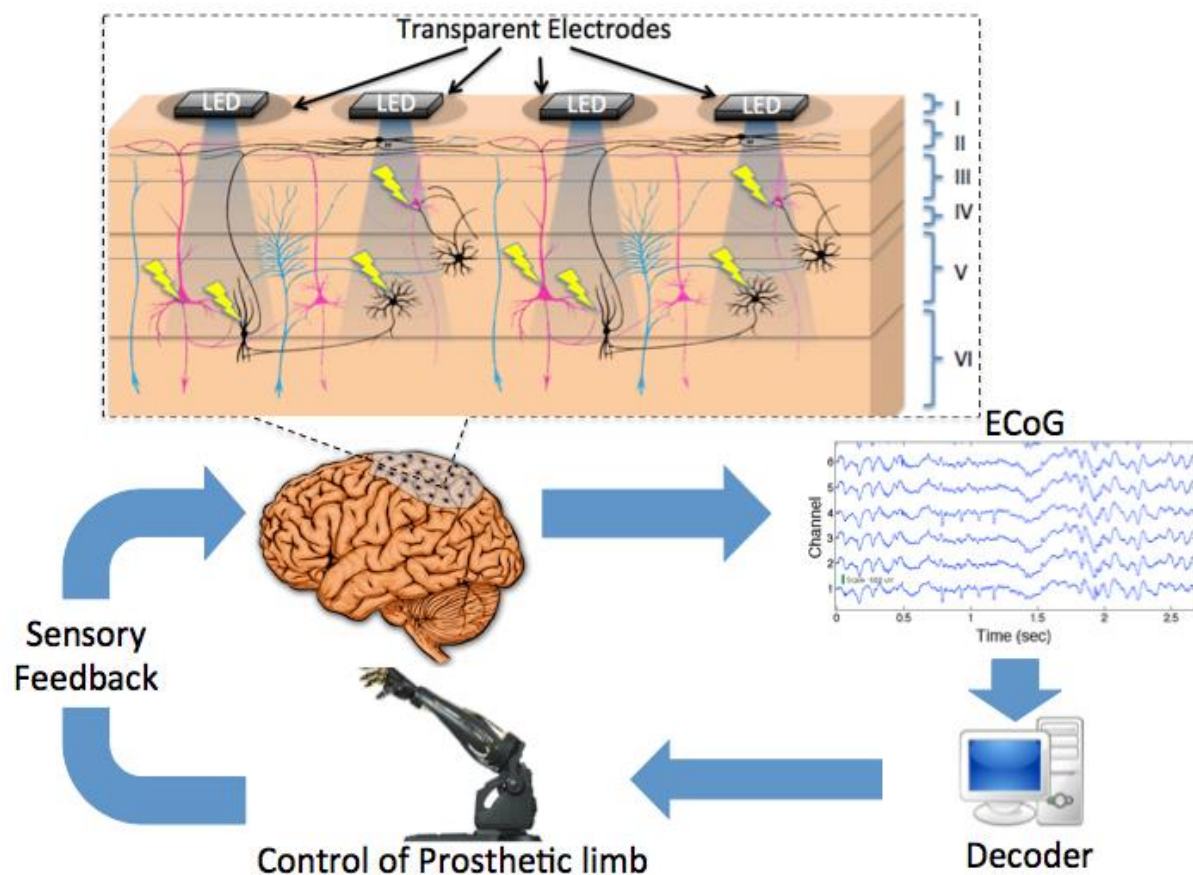


Figure 3-1. A concept diagram of BMI system with an Opto- μ ECoG array

3.2 Design

The concept of the Opto- μ ECoG array is illustrated in Fig. 3-2. Specific design considerations were made to address the above-mentioned limitations of the current optogenetics-based neural interfaces, as discussed below.

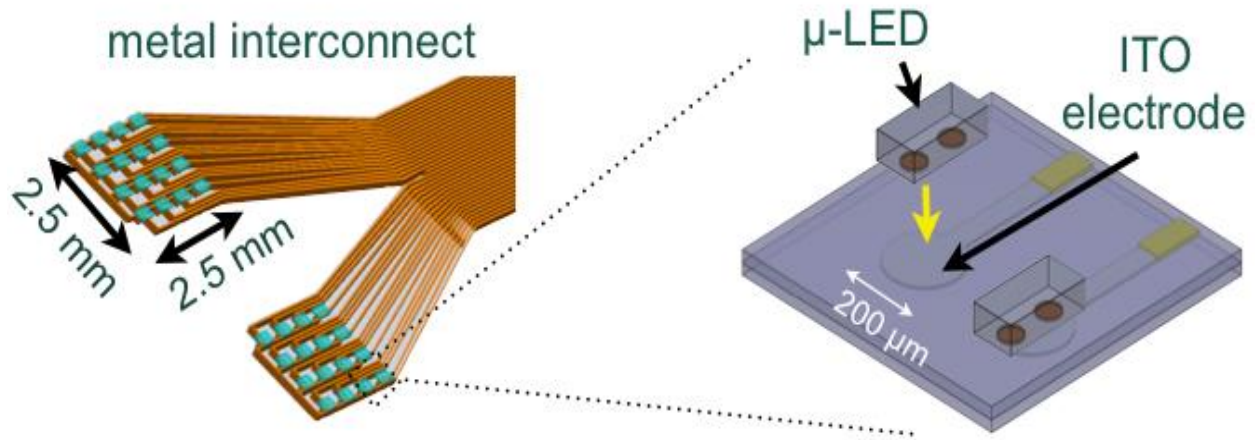


Figure 3-2. Concept illustration of the proposed Opto- μ ECoG array

3.2.1 A multichannel, bi-directional system

The Opto- μ ECoG array comprises two sub-arrays, each containing 16 channels (4×4) on a $2.5 \times 2.5 \text{ mm}^2$ footprint designed to match the bilateral hemispherical area of the visual cortices in rats. Each channel was constructed by assembling a surface mounted μ -LED ($220 \times 270 \times 50 \text{ }\mu\text{m}^3$, peak wavelength at 460 nm, Cree® TR2227TM, Cree, NC, USA) on top of the transparent electrode, to allow simultaneous optical stimulation and electrical recording. This system permits simultaneous recording and/or stimulation of neural activity in individual or multiple spots. LED light sources potentially could be powered and controlled through wireless telemetry links to achieve a truly untethered system.

3.2.2 Maximum target cortical area of the optical stimulation

Optically transparent epidural electrodes allowed maximum exposure of the target cortical area for light stimulation. The transparent electrodes were fabricated using indium tin oxide (ITO), a transparent conductive oxide (TCO) exhibiting low electrical resistivity and high transmittance in the visible spectrum [119]. ITO is considered not only the most promising electrode material for various optoelectronic applications [120], but also excellent biocompatible material, based on cytotoxicity and protein adsorption tests [121].

3.2.3 Maximum spatial resolution of optical stimulation and electrical recording

The LED array must cover the entire targeted cortical area, whereas the effective area of a single LED must be minimized to achieve high spatial resolution of the optical stimulation. Arrangement of the μ -LEDs was estimated based on the scattering property of blue light, which was investigated by quantifying the optical throughput of the LED in the brain tissue. Efforts have been made to increase spatial resolution of μ ECoG arrays, but no consensus has been reached on optimal size and spacing of the electrodes; such size and separation are major parameters requiring careful design to achieve high spatial resolution and signal-to-noise ratio (SNR) of the ECoG recording. In this work, the ECoG electrodes were designed with diameter 200 μ m and separation 700 μ m, based on a recent study by Slutzky et al. [122] of optimal spacing of subdural, epidural, and scalp electrodes.

3.2.4. Biocompatibility and minimum invasiveness

The micro-optoelectronic device must be biocompatible and flexible for chronic implantation. Parylene-C was used as the prime structural and packaging polymer, which has shown superb biocompatibility (ISO 10993 & USP Class VI), great flexibility, optical transparency, chemical inertness, and low permeability [97]. Epidural placement of the device on the cortex is less

invasive than intracortical penetrating electrodes, thus further reducing risk of brain tissue damage.

3.3 Fabrication

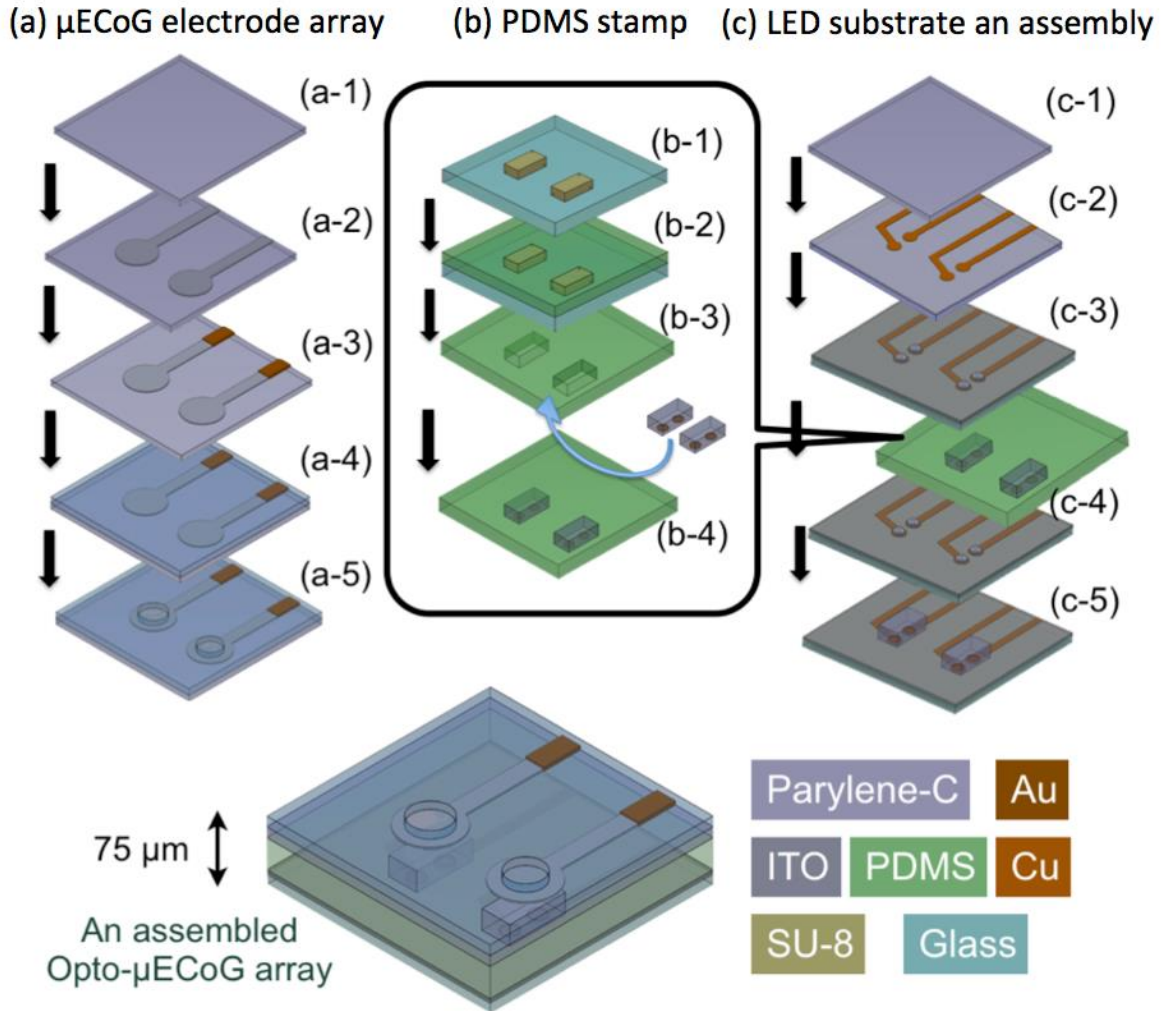


Figure 3-3. Opto- μ ECoG array fabrication process: (a) processes of μ ECoG electrode array fabrication; (b) PDMS stamp fabrication; and (c) LED array substrate fabrication and integration with the μ ECoG electrodes.

To reduce fabrication complexity, the ECoG array and the LED array were fabricated and calibrated separately. The system then was assembled by polymer bonding of individual components with SU-8. The detailed fabrication process was divided into three steps, as described below.

3.3.1 Fabrication of the μ ECoG electrode array

Devices were fabricated using a Parylene-metal-Parylene multilayer technique, as shown in Fig. 3-3. (a-1) A 3-inch glass carrier wafer was cleaned by sonication in acetone, isopropanol (IPA), and deionized (DI) water (3 mins each). Five (5) μ m Parylene-C was deposited on the glass substrate using a chemical vapor deposition (CVD) system (PDS2010 Parylene Coater, Specialty Coating System, IN, USA). (a-2) DC sputtering of a 0.1 μ m thick ITO layer was performed in a Kurt Lesker Axiss PVD System (a 2-inch diameter ITO target [10 wt% SnO₂], base pressure was 5e-6 Torr). The ITO target was oxidized by pre-sputtering with DC power of 50 W in an Ar- O₂ gas mixture (20 sccm of Ar and 1 sccm of O₂) for 5 mins. Then the ITO layer was wet-etched with 0.4 M of oxalic acid using a photoresist (PR) mask.

(a-3) A 0.1 μ m Au layer was deposited on the substrate by a thermal evaporator (Edward Auto306, Edwards, UK) and patterned by wet etching to form interconnection leads, as shown in Fig. 3-4b. (a-4) After that, another layer of Parylene-C was deposited to seal the entire structure. (a-5) Electrode sites and contact vias were opened by oxygen plasma etching of the Parylene.

3.3.2 Fabrication of a PDMS stamp

(b-1) A 3-inch glass wafer was cleaned and went through a dehydration bake. A $\sim$ 30 μ m SU-8 layer was spun onto the wafer and patterned as the mold for fabricating a PDMS stamp. (b-2) PDMS (Sylgard 184, Dow Corning, MI, USA) was poured over the SU-8 mold to form the stamp, which contained cavities matching the shape of the LED chip. (b-3) After curing for 40 mins at 95 °C, the PDMS stamp was peeled off the mold. (b-4) Thirty-two LED dies were aligned in the cavities of the stamp, metal pads facing outward. The assembly method using the PDMS stamp was adapted from [123], and the yield of the chip assembly was above 96%.

3.3.3 Substrate fabrication and assembly

(c-1) A 3-inch glass carrier wafer was cleaned by sonication in acetone, IPA, and DI water (3 mins each), followed by Parylene-C (5 μm in thickness) deposition. (c-2) A 0.3 μm Cu layer was deposited in the thermal evaporator and patterned by wet etching to form contact pads and interconnection leads. A PR mask was patterned selectively to expose only the metal contacts for LED connections. (c-3) Low melting point (LMP) solder (melting point at $\sim 62^\circ\text{C}$, 144 ALLOY Field's Metal, Rotometals, Inc., CA, USA) was applied on the contacts. The substrate was then rinsed with acetone, IPA, and DI water to remove the PR layer. (c-4) The PDMS stamp with the embedded LEDs was aligned to match the pre-soldered receiver sites on the substrate. (c-5) The substrate with the aligned PDMS stamp was heated on a hot plate at 90°C for 30 secs. The PDMS stamp was removed carefully after the substrate had cooled to 40°C , leaving the LEDs attached to the contact pads of the substrate. The substrate was then submerged into a hot acidic water bath (90°C , pH of 2.0) for 1 min. This process permits fine adjustments of LED alignment and the formation of an electrical connection in a self-assembly manner (Fig. 3-4d and 3-4e). (c-6) A $\sim 50\ \mu\text{m}$ SU-8 layer was spun on the μECoG array as a bonding adhesive. The assembled LED array was aligned and bonded to the μECoG array, followed by oxygen plasma etching to define the contour of the carrier substrate.

3.4 Results and discussions

Fig. 3-4 shows an assembled Opto- μECoG interface, with total thickness $\sim 75\ \mu\text{m}$. Several experiments were conducted to characterize the optical, electrical, and thermal properties of the fabricated devices. *In vivo* acute animal experiments were performed to demonstrate the ability of the Opto- μECoG interface to simultaneously modulate and record neural activities in the visual cortex of a rat.

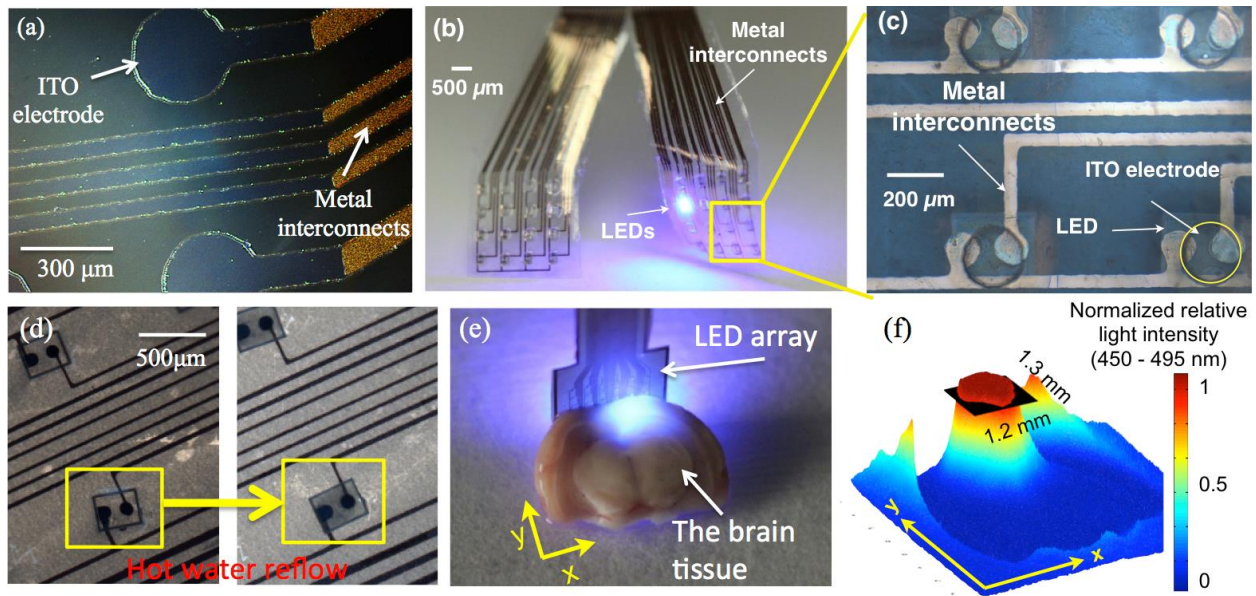


Figure 3-4. A fabricated Opto- μ ECoG array and light scattering test: (a) ITO electrode array, (b) the assembled Opto- μ ECoG array, and (c) zoomed-in image of individual modules. (d) LED array before hot water reflow (left) and after the reflow (right). (e) Light scattering property of the μ -LED chip was estimated in brain tissue and (f) a normalized relative light intensity was estimated. Both color and the height (z-axis) indicate blue light intensity.

3.4.1 Optical properties

In this study, 1000-Å-thick ITO was sputtered on a 10- μ m-thick Parylene-C film at room temperature. Optical transmittance of the ITO-Parylene film was measured over the visible wavelength range (450 – 750 nm) at 37 °C using a custom-built spectrum analyzer. Average transmittance throughout the spectrum was $\sim 80\%$, while transmittance at the wavelength of 470 nm was $\sim 55\%$ (Fig. 3-5a). This result was comparable with the $\sim 60\%$ transmittance measured using a 10- μ m-thick Parylene-C film reported in [124], considering the additional light absorption of the Parylene-C film.

To optimize the design of the LED array for maximizing the effective stimulation area while minimizing the number of LED chips, the optical throughput of the LED chip in brain tissue was studied. The LED array was placed on top of the harvested rat brain tissue, and the

images of scattered light from an activated LED were captured (Fig. 3-4e) and analyzed using MATLAB (MATLAB 2013, MathWorks, MA, USA). Relative light intensity across the brain tissue was labeled with different colors and plotted in Fig. 3-4f. The LED chip was driven by 2.8 V, resulting in a power consumption of 21 mW, a temperature increase of 0.3 °C, and irradiance of 10 mW/mm². The effective area, where 90% of optical output was delivered, was estimated to be ~ 1.2 mm in horizontal distance and ~ 1.3 mm in depth. Considering the scattering property of the LED and the 55% transmittance rate of an ITO-Parylene film at 470 nm, the maximum radius of effective light excitation was estimated to be approximately 300 – 350 µm. Consequently, the minimum inter-electrode/LED spacing was designed to be 600 – 700 µm.

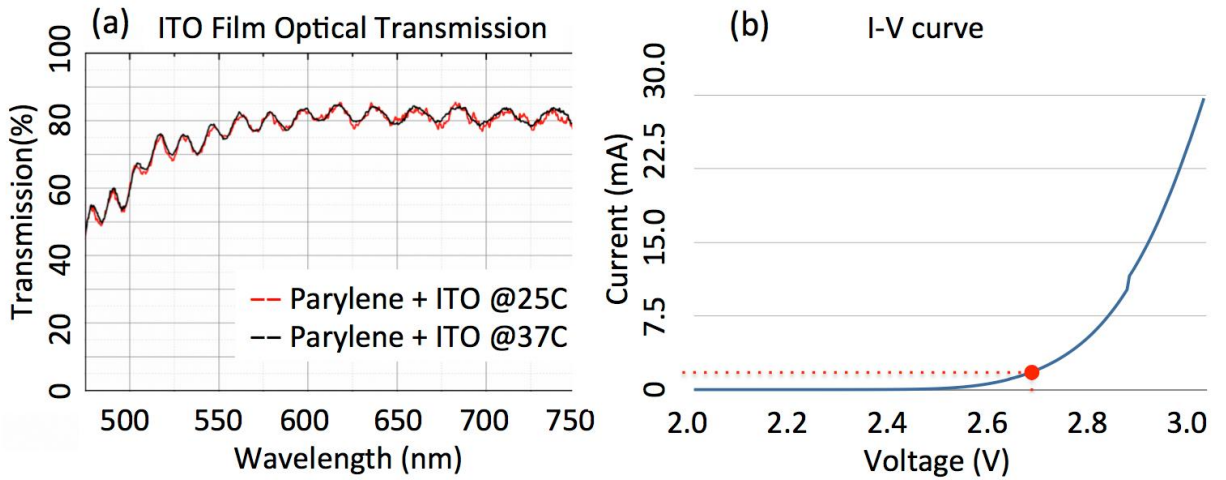


Figure 3-5. Optical and electrical properties: (a) visible transmission spectra of ITO film and (b) I-V characteristic of a µ-LED chip.

3.4.2 Electrical properties

Sheet resistance of the ITO film was measured by a 4-point-probe unit (VEECO FPP-5000, NY, USA). The pre-oxidation step described in the fabrication process significantly reduced the sheet resistance of ITO films from 88 Ω/sq to 12 Ω/sq, without compromising their optical transparency [125]. To demonstrate the benefit of the multilayer metal interconnects, the electrode-electrolyte interface impedances of electrode arrays with only ITO metalization as well

as arrays with both ITO electrode and ITO-Au interconnect metallization was tested. Measurements were done at 1 kHz using a built-in electrode-impedance-testing circuitry in Intan evaluation board (RHA2116 and RHA2000-EVAL, Intan Tech. LLC, CA, USA). Arrays with ITO-Au interconnect showed lower impedances ranging from 1 to 5 k Ω , as compared to the impedances of arrays with only ITO metalization (7 – 20 k Ω). These results demonstrated that the ITO-metal multilayer metalization not only prevented breakage of ITO leads due to the brittleness of ITO [117], but also significantly reduced overall impedance of the electrodes.

Power consumption of the μ -LED at various operating conditions was investigated also, which provides a useful guideline for optical stimulation in *in vivo* experiments and future integration of the LED array with wireless power telemetry. The nonlinear current-voltage characteristic of a single μ -LED was measured with an impedance analyzer (HP 4191A RF, Hewlett Packard, CA, USA), as shown in Fig. 3-5b. Since a minimum light-energy density of 1 mW/mm² must be delivered onto the target area to induce action potentials, a minimum input voltage of ~ 2.7 V is required to drive a single μ -LED chip, resulting in a total power consumption of 3.4 mW.

3.4.3 Thermal properties

Tissue damage caused by heat production of an implanted light source is a major concern, since the epidurally-implanted LED array will interface directly with brain tissue. Therefore, understanding thermal properties of the LED chip under various conditions is critical.

In this work, temperature variation of the LED array was investigated using a high definition infrared camera (Delta Therm. HS1570 and software (DT v.2.19)) with different input voltages (2.7 – 3.2 V) and activation durations (10, 50, and 100 ms, respectively). Thermal images were taken in a room (at room temperature of 22.7 °C) where ventilation was strictly

controlled to minimize ambient thermal noise, and the array was suspended in the air during the LED activation. Captured thermal images were calibrated and analyzed using MATLAB; results are plotted in Fig. 3-6. As expected, both the input voltage and duration of stimulation dramatically changed the temperature profile of the LED. With the lowest input voltage of 2.7 V, the heat produced by the LED resulted in the local temperature increase of less than 0.1 °C.

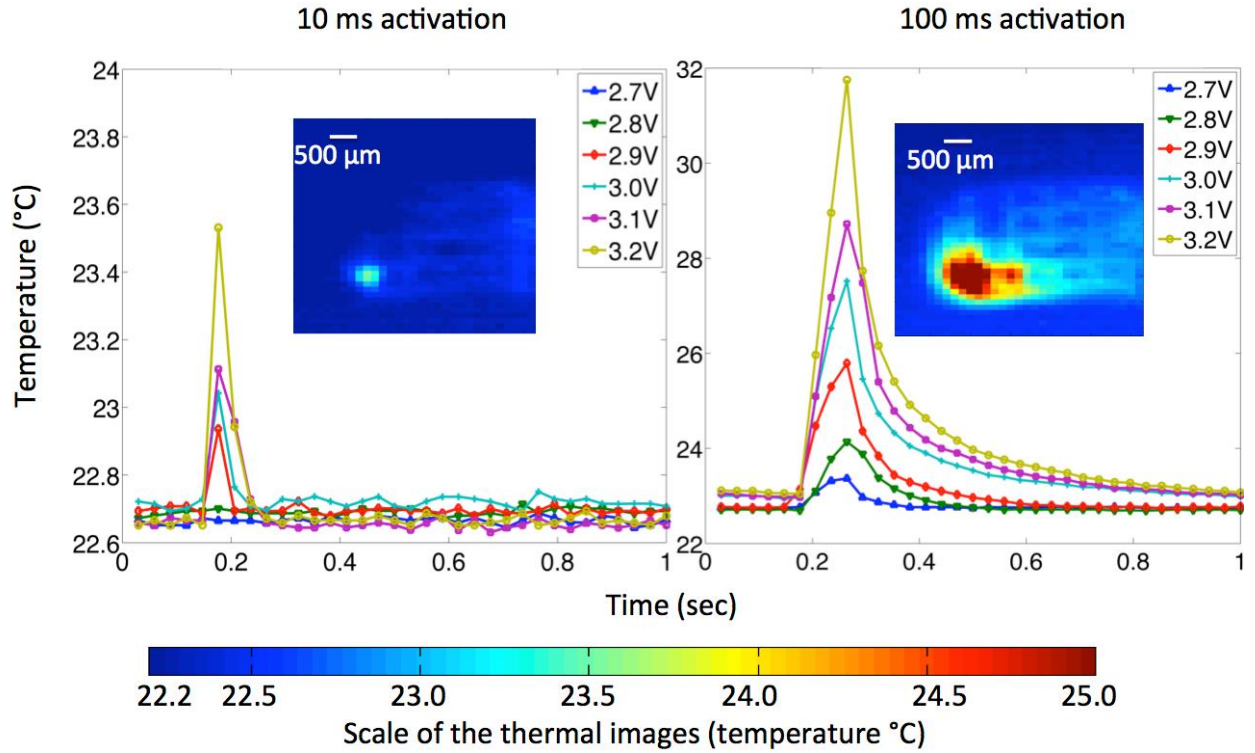


Figure 3-6. Thermal properties: Heat dynamics of the Opto- μ ECoG array at various input voltages (2.7 – 3.2 V) for 10 ms (left) and 100 ms (right) activations. The insets show corresponding thermal images of a single LED activation taken at the maximum temperature with 3.2 V input voltage and activation durations of 10 ms and 100 ms, respectively.

Based on prior work by Andersen et al. [126], 0.5 °C is a conservative limit on temporarily-allowable temperature rise in brain tissue adjacent to the device. The test results suggest that a short pulse of activation (2.7 V amplitude and 10 – 100 ms pulse durations) would satisfy this safety limit. The heat dissipated within 50 ms and the LED temperature returned to the baseline point (~ 22.7 °C). As the input voltage and the activation duration increased,

significant heat was generated by the LED, resulting in higher local temperature, larger heat conduction, and longer heat dissipation time. Maximum temperature increase was over 9 °C when the LED was driven by 3.2 V with the 100 ms activation duration. Considering the tradeoff between heat production and light-energy density of the LED, the 2.7 V input voltage and 100 ms duration was chosen as the stimulation parameters for *in vivo* animal experiments.

3.4.4 Soak testing

A passive soak testing also was conducted to study electrode array reliability. During experiments, the device was immersed in saline at an elevated temperature of 37 °C, then removed daily from the oven to monitor device failures through measurement of electrical function and visual inspection. The device was suspected to fail from soak testing when its impedance was over 1 MΩ. Then the array was tested in saline where a short pulse with 1 mV amplitude and 10 ms duration was injected. If the signal-to-noise ratio (SNR) of recording through the electrode was lower than 1 dB, the electrode was confirmed being failed. Three of 30 electrodes failed after 25 days of soaking. From microscope inspection, all 3 failures were caused mainly by delamination and/or water traps at the Parylene-metal interface due to insufficient Parylene sealing on the periphery of the electrodes. The 27 electrodes remained functioning (impedance: 15 – 110 kΩ) even after 25 days of soak testing (Fig. 3-7).

3.4.5 *In vivo* animal experiments

In vivo acute animal experiments were conducted to evaluate surgical and functional applicability of the devices at the cortical interface. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University. Prior to the acute experiments, rodent subjects (Sprague-Dawley rats: 250 – 400 g) had neurons in their

primary visual cortex (V1) transfected with ChR2 to enable light sensitivity. For the viral transduction, each rat was anesthetized with a mixture of ketamine and xylazine and placed in a

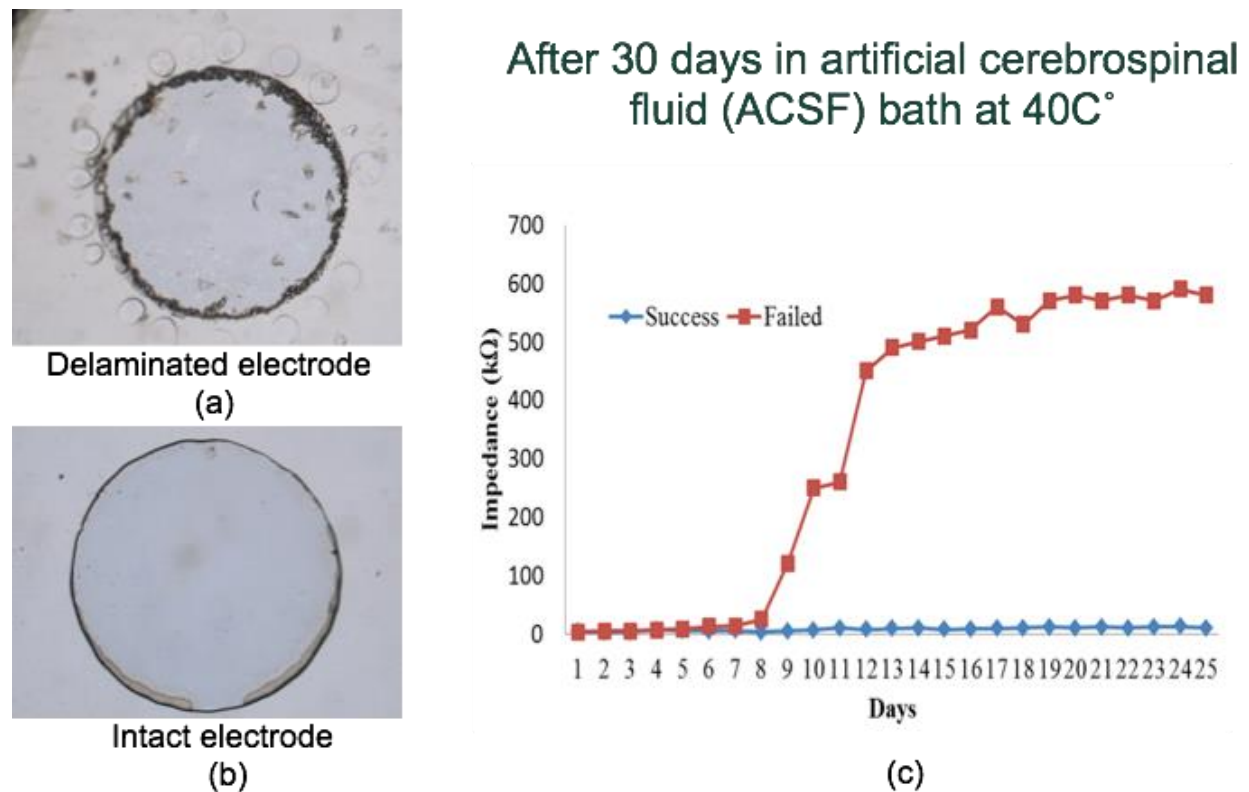


Figure 3-7. Soak testing result for 25 days: (a) Impedance changes in failed and successful electrodes during the 25 days of soak testing. The impedance of the failed electrode increased significantly at day 9. Optical images of (b) successful and (c) failed electrodes are shown. 3 out of 30 electrodes were failed during the soak testing. The main cause of the failure was delamination due to the insufficient protective sealing of Parylene on the periphery of metal electrodes.

stereotaxic apparatus. Using sterile surgical procedures, a 3 – 4 cm incision was made in the skin overlying the skull and a small region of bone was removed to expose V1. Using a glass pipette and a pressure injection system, 1.0 μl ($10 \times 10^{11} \sim 10 \times 10^{12}$ vector genome (vg)/mL) of the virus solution (AAV-hSyn-hChR2(H134R)-mCherry; UNC Vector Core, NC, USA) was injected at three to four equidistant locations. Injections occurred over a 2 – 3 minute period, and the glass pipette was left in place for an additional 10 minutes to allow the vector to diffuse away from the injection site. The cortex then was covered with Gelfoam and the skin overlying the

skull was sutured closed. The animals were allowed to recover from anesthesia, housed separately, and given pain medication and antibiotics to minimize infection.

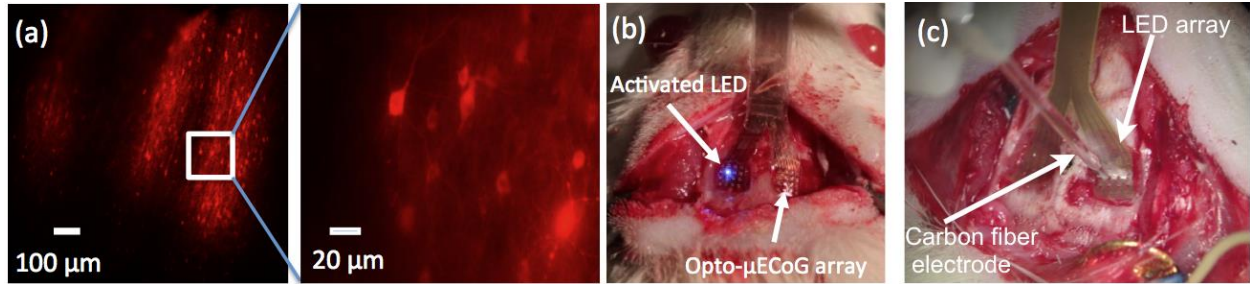


Figure 3-8. *In vivo* experiments: (a) mCherry fluorescence in a coronal slice of the transfected rat V1. *In vivo* recording set-ups for (b) optical neural modulation and electrical recording of the Opto-μECoG array and (c) the light-evoked extracellular recordings using the epidural LED array.

For the device implant, the transfected (2 - 4 weeks after the injection) rat was re-anesthetized and placed back into the stereotaxis. The developed Opto-μECoG array was surgically implanted over V1 and the tests were conducted, as described below.

The capability of simultaneous optical neural modulation and electrical recording of the Opto-μECoG array was investigated. In this case, the μ-LED array was placed on rat V1 (Fig. 3-7b) to optically modulate the neural activities with a light power density of $\sim 1 \text{ mW/mm}^2$ and a light pulse duration of 100 ms. ECoG signals induced by the LED light stimulation were simultaneously recorded through the selected 15 ITO electrode channels. Recorded signals were amplified using the RHA2000 amplifier array, then analyzed using MATLAB and Chronux toolbox (Chronux.org). Fig. 3-9 shows representative results obtained when the μ-LED of channel 6 was activated with an input voltage of 2.7 V at 1 Hz for 100 ms. The corresponding light-evoked neural activity was translated into the time-varying energy distribution in the gamma band (50 – 180 Hz) as shown in Fig. 3-8a. To study temporal and spatial characteristics of the captured neural activity, a matrix of the average gamma band energies of the 15 channels was generated every 50 ms after optical stimulation (Fig. 3-9c). In the first 50 ms window, the

highest gamma band power was observed at channel 6 where the light source was located. As time passed, energy spread to neighboring channels. This preliminary result suggests that epidural cortical stimulation by the LED array is capable of optically modulating cortical neural activity.

To verify activation of transfected neurons located in deep cortical layers following optical stimulation directed at the cortical surface, extracellular recordings were performed using a carbon fiber probe (Carbostar-1, impedance range of 100 – 200 k Ω , Kation Scientific LLC, MN, USA) at various cortical depths. To achieve this, a polyimide μ -LED array was prepared as described in [30], only now with holes ($\sim$ 200 μ m in diameter) in spaces between μ -LEDs. Once the μ -LED array was placed on V1, the carbon fiber probe was inserted into the hole next to the activated μ -LED (Fig. 3-8b). While optically stimulating the superficial layer of V1 with an input voltage range of 2.7 – 3.2 V and a light pulse width of 50 – 300 ms, the light-evoked neural activity was monitored through the carbon fiber electrode controlled by a micropositioner (Model 2670, David Kopf Instruments, CA, USA) at different depths. Recorded data were pre-processed using NeuroQuest, a MATLAB tool box for neurophysiological signal processing, and light-evoked action potentials at different cortical layers of V1 were plotted (Fig. 3-9d) [127].

Clear spikes were observed at depths of 200 – 600 μ m in response to the optical stimulation, whereas no neural activity corresponding to the optical stimulation was observed at a depth of 700 μ m. After the *in vivo* experiment, the animals were perfused and the brain tissue harvested for histological analysis to verify actual penetration depth and the thermal effect of LED on brain tissue. This acute experiment revealed that epidural light stimulation using μ -LEDs was capable of evoking not only neurons in superficial layers (100 – 300 μ m) but also neurons in deep cortical layers (300 – 600 μ m).

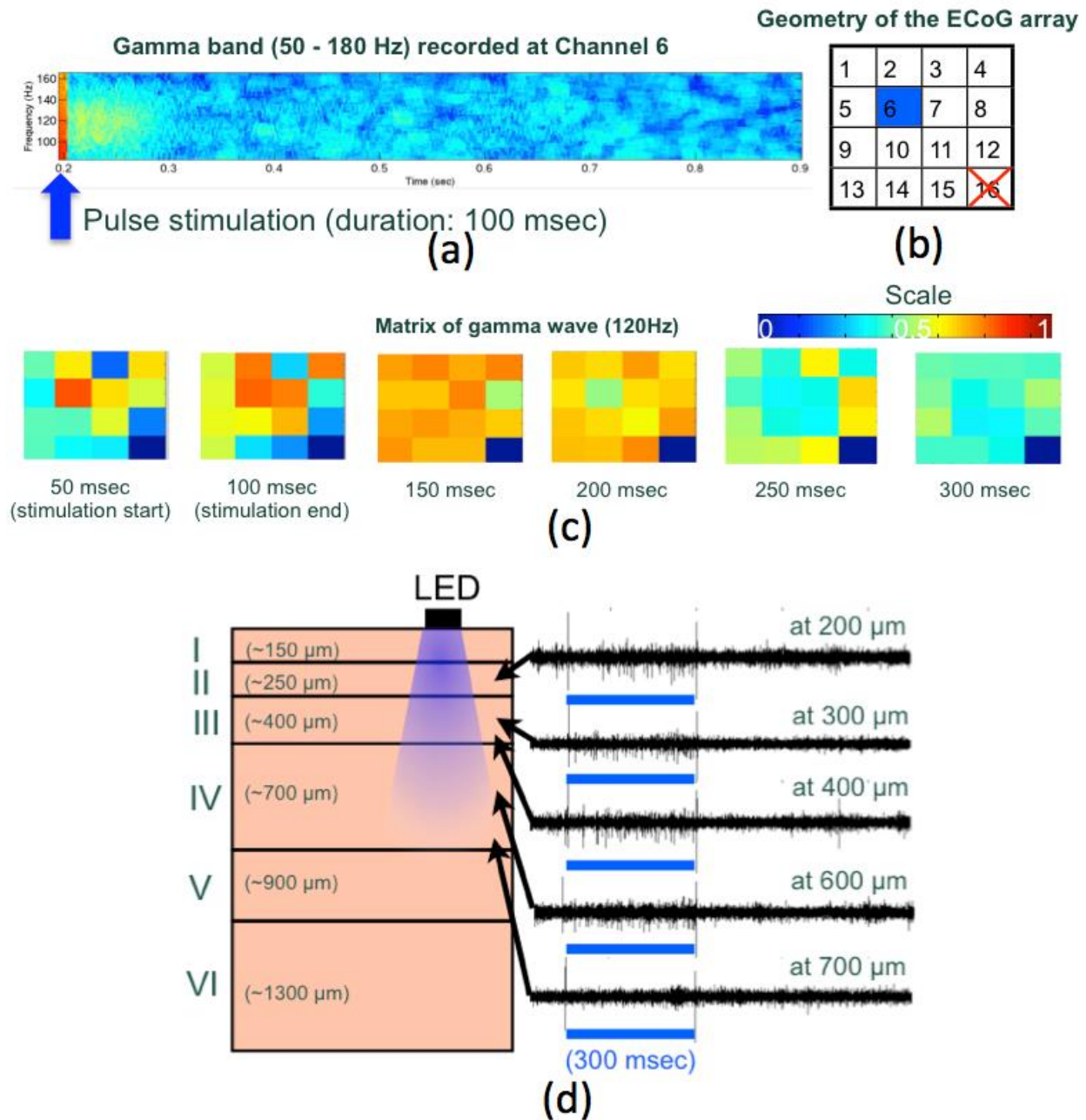


Figure 3-9. *In vivo* neural recording analysis results: (a) the spectrogram (50-180 Hz) of the optically evoked ECoG signal recorded through the selected channel (Ch 6) where the activated LED was located as shown in (b). (c) A series of a matrix of the average gamma band energy with 50 ms interval. (d) Light-evoked extracellular recordings at different depths through the carbon fiber electrode.

3.5 Conclusion

In this chapter, this author designed, fabricated, and characterized the Opto- μ ECoG array capable of simultaneous optical stimulation and electrophysiological recording of cortical neurons. The proposed hybrid neural interface had four unique features to address major challenges of existing optogenetics-based BMIs: First, the multichannel LED array allowed the possibility of achieving a truly untethered system crucial for chronic implants. Second, the optically transparent ITO epidural electrodes enabled maximum exposure of the target cortical area for optical stimulation. Third, integrated ITO electrodes and LED light sources carefully were arranged to avoid redundant neural recordings and overlapping light stimulation due to closely-placed neighboring electrodes and μ -LEDs, respectively. Finally, polymer packaging and the epidural placement of the device on the cortex enabled long-term biocompatibility of the device and minimized risk of potential tissue damage. Thermal characteristics of the device indicated the critical range of stimulation parameters, such as input voltage and duration, so as to avoid brain tissue damage from overheating. Optical and electrical properties of the device fulfilled design requirements for an epidural optical neural modulator.

Short-term stability of the device was demonstrated using soak testing. *In vivo* acute experiments verified the high-quality recording potential, and the possibility of chronic implantation. This author also demonstrated for the first time that epidural LED light stimulation could evoke neural activity not only within the superficial cortical layers, but also in deeper cortical layers (to a depth of $\sim 600\ \mu\text{m}$).

Although the thermal property of the array described in section 3.4.3 suggests that the LED input voltage of 3.2 V for 300 ms results in local temperature increase of over 0.5 °C, no significant tissue damages due to the thermal effect were observed. Since these thermal images

were collected without consideration of thermoregulation of cerebral circulation in the brain, more systematic investigation must be done to determine actual thermal properties of the implanted array.

Artifacts were observed during *in vivo* ECoG recording when the LED was powered on. These artifacts are believed to be light-induced artifacts, also known as the Becquerel photovoltaic effect. One possible solution to address this issue is to insert an extra layer of ITO shield between electrode and LED arrays, which effectively can eliminate light-induced artifacts and any electrical artifacts, as demonstrated by Zorzos et al. [128].

This novel opto-neural interface will permit multichannel, bi-directional interaction with neurons at a level of sophistication not possible with existing optogenetics instrumentation, thus lays the foundation for the development of advanced optogenetics-based BMIs. From system design perspectives, this study has shown that LED input power of ~ 3.4 mW is sufficient for activation of ChR2 expressing neurons. This makes it possible to drive the LED wirelessly with suitable designs of inductive telemetry [76]. As an example of future chronic implants, it is estimated that the 32-channel Opto- μ ECoG system would require a total input power of ~ 54.4 mW to permit optical stimulation through 16 LEDs simultaneously on one side of the bilateral hemispherical cortex. Simultaneous recording of neural activities from 16 electrodes will consume less than 9 mW, estimated from the power dissipation of Intan RHD 2000-series chips. It is envisioned, approximately, that the proposed system will require a total power of < 65 mW transferred through inductive coupling, with a coil separation of 1-3 cm in a rat model. Other possible power sources may include small rechargeable batteries and energy-scavenging devices. The effort to realize a fully implantable wireless neural interface is continued in Chapters 7 and 8.

CHAPTER 4

Three-dimensional polymer waveguides array for optogenetics

4.1 Motivation

In the previous chapter, an epidural optical stimulating approach using the Opto- μ ECoG array, a transparent μ ECoG electrodes array with integrated μ -LEDs, was introduced. While the 32 μ -LEDs array significantly improved the spatial resolution of the optical stimulation, its depth resolution had a severe limitation. More, the depth control of the epidural optical stimulation required relatively high input voltage and power to deliver light to deep cortical layers. Finally, selective stimulation of the target layer was impossible with the surface light source.

This chapter presents the design, fabrication and characterization of two types of fully implantable, individually addressable, three-dimensional (3-D) micro-waveguides (μ -waveguides) array for optogenetics. Integration of individually addressable μ -LED chips with μ -waveguides, shown in Fig. 4-1, enables minimization of the scattering of LED lights in the brain tissue to achieve high spatial resolution, and precise light delivery to target neurons. The array contains 32 embedded μ -LED light sources on a polyimide substrate. Polymer μ -waveguides are fabricated separately on a PDMS substrate using a backside exposure technique and bond with the multi-LED array using a shape-matching assembly. Physical dimension of the array is $2.5 \times 2.5 \text{ mm}^2$ with 16 (4×4 grid) channels per each hemisphere to meet specifications of bilateral visual cortices in rats. Integrated light sources allow for the implementation of a truly untethered system, crucial for chronic implant in freely behaving animals.

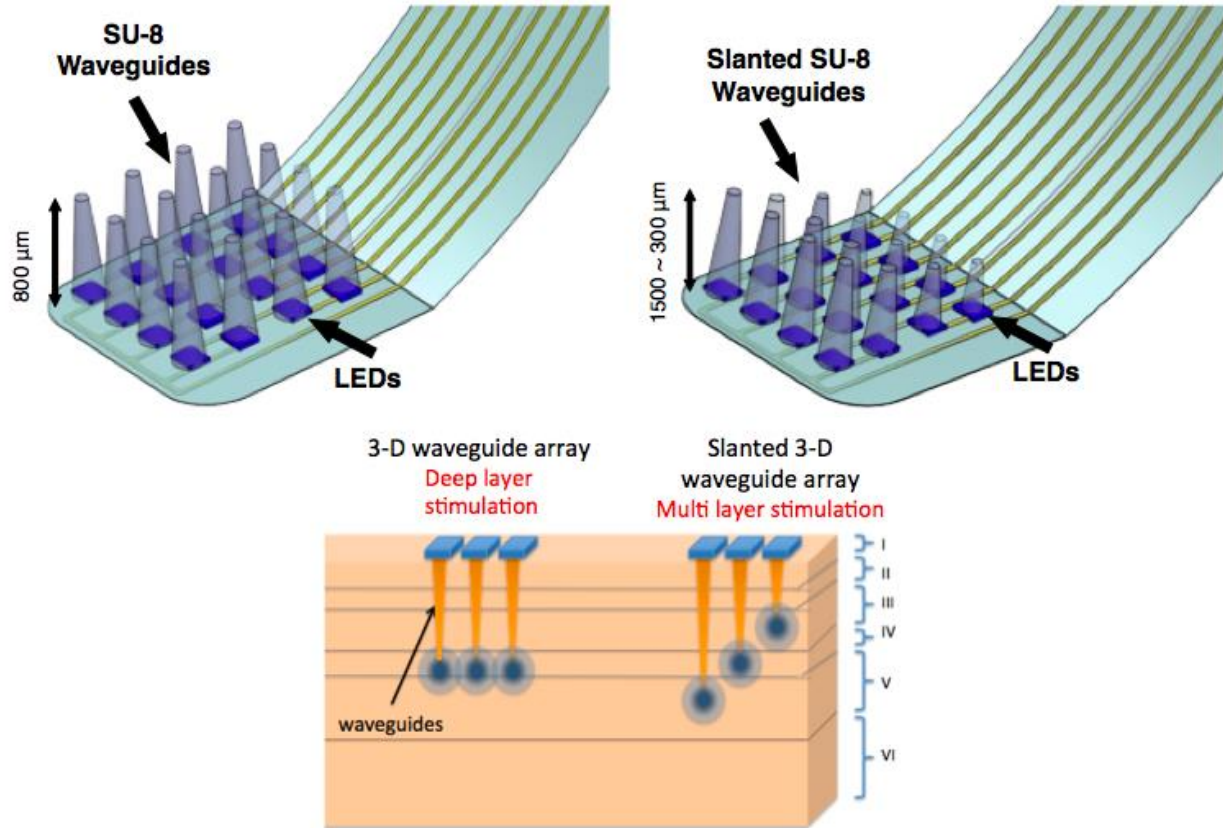


Figure 4-1. Conceptual illustration of the multi-LED array with 3-D polymer waveguides arrays for deep brain optical stimulation.

The spatial resolution of the 3-D μ -waveguides array is further improved by proposing a slanted waveguides array. The length-varying microneedle structure allows precise light delivery to the target neurons in individual cortical layers simultaneously. Several approaches for making a slanted microneedle structure have been reported, but they either required a specialized machine and material [25], or lacked control of the microneedle size that determines light coupling efficiency [129]. A new technique, “droplet backside exposure” (DBE) utilizing height variance in the profile of a droplet, was developed to create the slanted microneedles array.

The DBE method uses surface energy differences between two adjacent contact areas of a PDMS substrate to define the curvature of a droplet. As shown in Fig. 4-2, a dome-shaped SU-8 droplet with a designed base size can be formed on a patterned hydrophilic area (O₂ plasma

treated-PDMS surface) with hydrophobic surroundings (intact PDMS surface). In a certain volume range, the droplet is confined within the boundary between the hydrophilic and hydrophobic regions, due to differences of surface energies, and this allows variant heights of droplet/s by controlling the SU-8 volume. Then the slanted microneedles structure can be formed in the dome structure using a backside exposure [130]. Details of the DBE method are reported in Chapter 5.

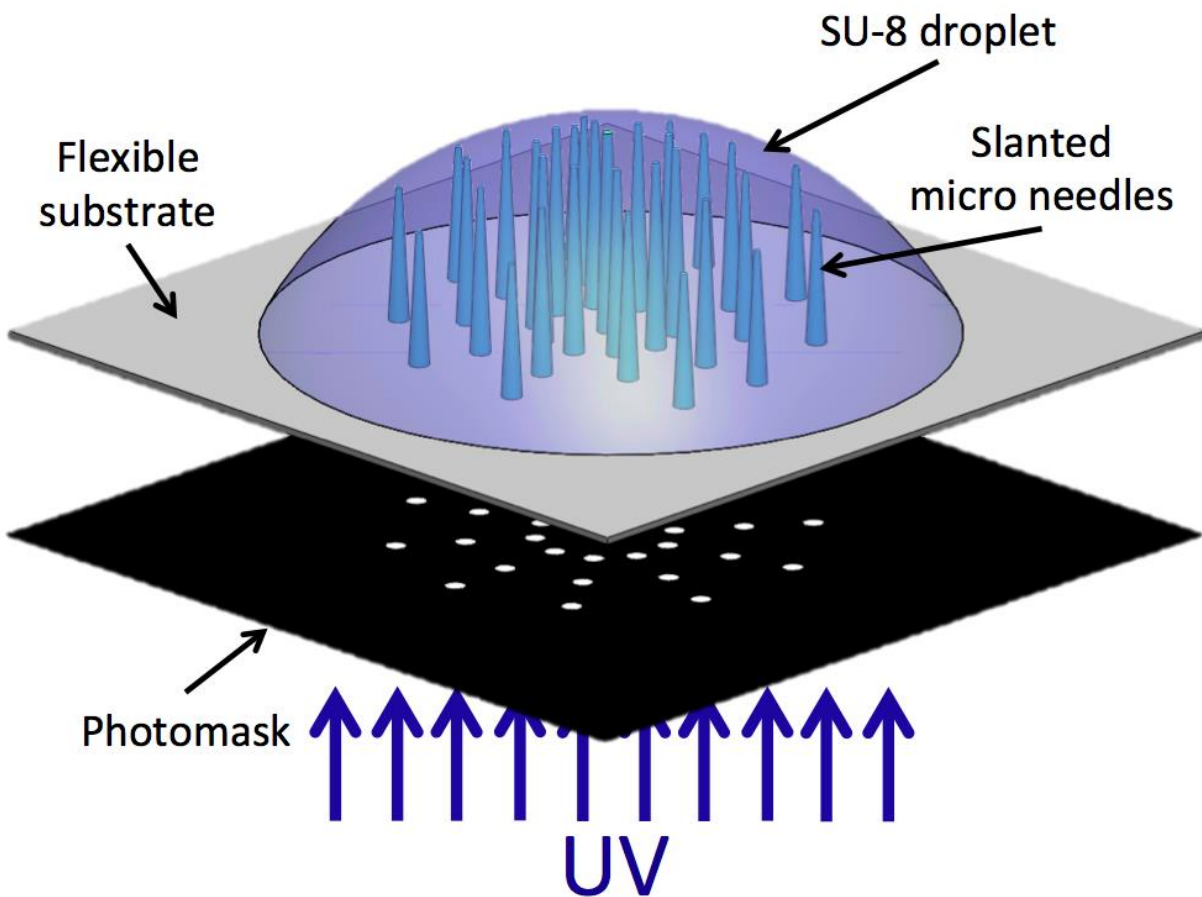


Figure 4-2. Principle of the droplet backside exposure (DBE) method

This chapter first describes design specifications of 3-D waveguides arrays, followed by devices fabrication. Next, the optical, electrical, and mechanical characteristics of the fabricated

device are investigated. Finally, the *in vivo* experiments are performed to investigate long-term biocompatibility and reliability of the proposed devices.

4.2 Design

Several design objectives were considered for optical stimulation arrays. First, the μ -waveguide must be able to deliver sufficient light to the desired cortical layer. The 3-D μ -waveguides array was designed to target cortical layer IV in primary visual cortex (V1) of the rat. Layer IV in V1 is an important area to study the connection between the visual cortex and other deep brain structures, since V1 receives most visual input from the lateral geniculate nucleus (LGN) through layer IV. Because layer IV in V1 is located at a depth range between $\sim 600 \mu\text{m}$ and $\sim 1000 \mu\text{m}$, the waveguide length was designed to be $800 \mu\text{m}$. The slanted waveguides array was designed with lengths ranging from 400 to $1000 \mu\text{m}$ to provide access to multiple cortical layers (layers III to V) in the rat's primary visual cortex (V1). For certain applications of central nervous system (CNS) stimulation, delivering optical stimulation at various cortical layers is critical. Moreover, to restore motor and sensory functions, peripheral neuroprosthetic interface requires accessing multiple independent motor neuron subpopulations in peripheral nerves or muscles.

Second, the μ -waveguide must be capable of evoking neural activity at the target cortical region. This is related to coupling efficiency of the μ -waveguide. Typically $1\text{mW}/\text{mm}^2$ of throughput power is required to activate ChR2 expressing neurons. Butt coupling was utilized to pair diffuse light sources (μ -LEDs) and μ -waveguides. A diffuse source means that the source emits light in all directions and that the intensity of light is independent of direction. Butt coupling is an attempt to capture emitted light before it diverges outside the fiber core radius, so this simple coupling technique provides near optimal coupling efficiency with the size of the

fiber core smaller than the source radius [131]. To maximize coupling efficiency, the base diameter of the waveguide was designed to be 300 μm , covering most of the surface area of the light source ($220 \times 270 \mu\text{m}^2$).

Third, the μ -waveguide should be mechanically durable to penetrate brain tissue without breaking, while minimizing damage to brain tissue. A needle-shaped configuration - sharp tip and large supporting base - was selected as a basic structure.

Finally, light sources must be addressable individually and distributed over the targeting cortical area. Surface mounted μ -LED chips ($220 \times 270 \times 50 \mu\text{m}^3$, wavelength peak at 460 nm, Cree® TR2227TM, Cree, NC, USA) coupled with the waveguide allow precise optical stimulation in an individual or multiple spots simultaneously.

Within many available fabrication methods, polymer (SU-8)-based fabrication technique utilizing backside exposure lithography was considered, because of the high refractive index of SU-8, simplicity of fabrication technique, and biocompatibility suitable for bio-MEMS applications [132]. While the adapted method allows length control with different size of mask, independent control of dimensions (e.g. base and tip sizes, height) of the microneedle is not possible [132]. Control of these parameters is critical in waveguide design, since these parameters determine coupling efficiency, irradiance and total flux of the needle-shaped waveguide [31]. The DBE technique allows to control lengths, tip and bottom diameters of individual microneedles without sophisticated equipment and complex microfabrication. Moreover, it is relatively simple with only slight changes from regular lithography processes, and permits wafer-level microfabrication for large scale, high-density system integration.

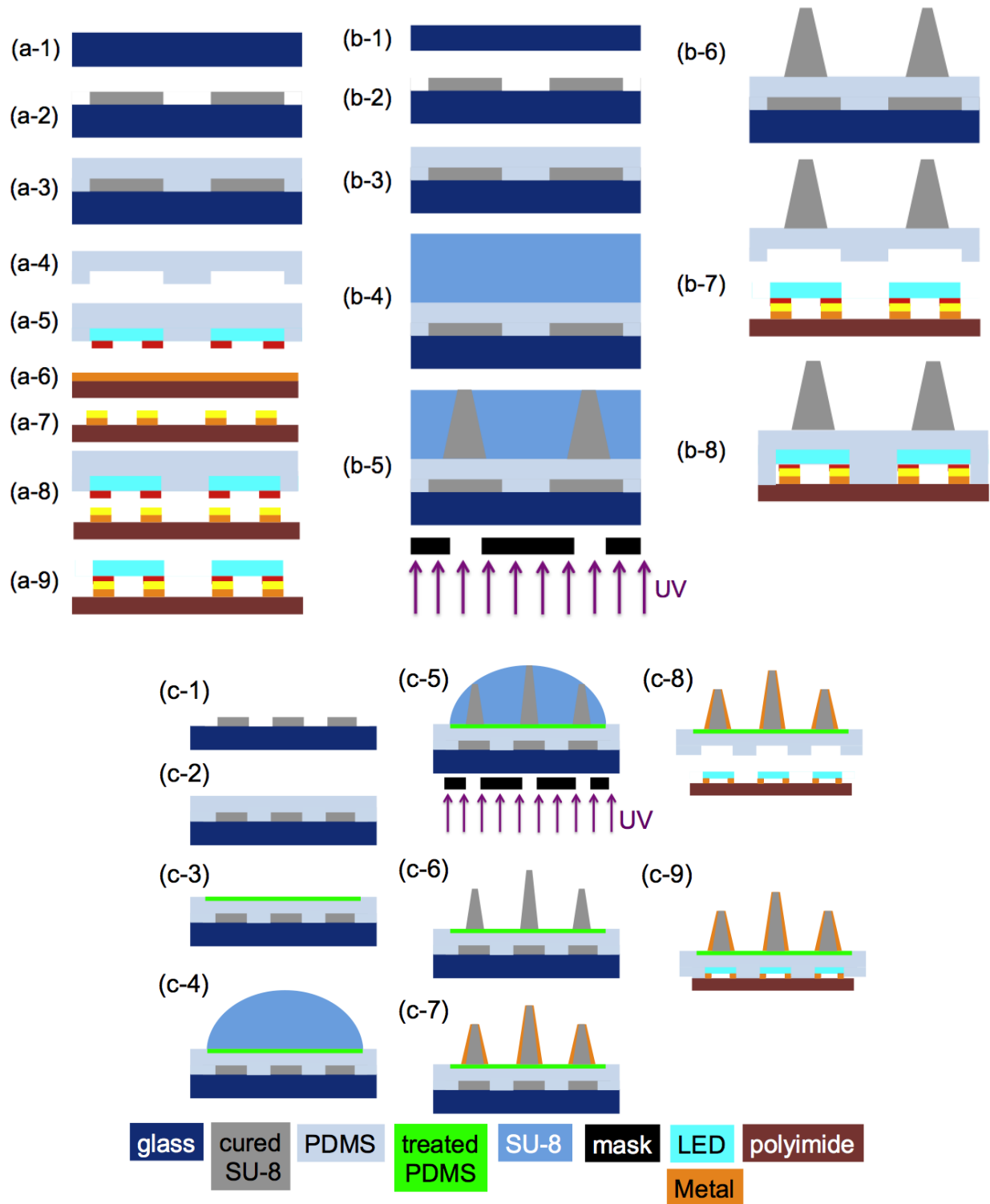


Figure 4-3. Fabrication process of (a) multi-LEDs array, (b) 3-D μ -waveguides array, and (c) slanted μ -waveguides array

4.3 Fabrication

To reduce fabrication complexity, the multi-LEDs array and the μ -waveguides array were fabricated and calibrated separately. Then the system was assembled by polymer bonding of individual components with SU-8. Detailed fabrication was divided into two steps and described in the following sections (Fig. 4-3).

4.3.1 Multi-LEDs array fabrication

Fig. 4-3a depicts the core fabrication process flow of the multi-LEDs array assembly method. Specifically, (a-1) A 3" silicon wafer was cleaned. (a-2) $\sim 30\ \mu\text{m}$ SU-8 layer was spun onto the wafer and patterned as the mold for fabricating a PDMS stamp. (a-3) PDMS was poured over the SU-8 mold to form the stamp, which contained cavities matching the shape of the LED chip. (a-4) After curing for 40 mins at $95\ ^\circ\text{C}$, the PDMS stamp was peeled from the mold. (a-5) 32 LED dies were aligned in the cavities of the stamp with metal pads facing outward. A substrate for LED assembly was fabricated using Pyralux®AP (AP7163E, DuPont, DE, USA). (a-6) 3" Pyralux® wafer was cut and cleaned, followed by Cu wet-etching. (a-7) Low melting point (LMP) solder (melting point at $\sim 62\ ^\circ\text{C}$, 144 ALLOY Field's Metal, Rotometals, Inc, CA, USA) was applied on the contacts. (a-8) The PDMS stamp with the embedded LEDs was aligned to match the LEDs' metal contacts with pre-soldered receiver sites on the substrate. (a-9) The substrate with the aligned PDMS stamp was heated on a hot plate at $90\ ^\circ\text{C}$ for 30 secs. The PDMS stamp was peeled off carefully after the substrate was cooled to $40\ ^\circ\text{C}$. The substrate, with the attached LEDs, was then submerged into a hot acidic water bath ($90\ ^\circ\text{C}$, pH of 2.0) for 1 min. This process permits fine adjustment of LED alignment and the formation of electrical connection in a self-assembly manner [123]. After that, another layer of Parylene-C was

deposited to seal the entire structure, and contact vias were opened by oxygen plasma etching of the Parylene.

4.3.2 A 3-D μ -waveguides array fabrication and assembly

Fig. 4-3b depicts the fabrication process flow of the 3-D μ -waveguides array fabrication and assembly method. Specifically, (b-1) a 3" glass wafer was cleaned and put through a dehydration bake. (b-2) a $\sim 50\ \mu\text{m}$ SU-8 layer was spun onto the wafer and patterned as the mock LEDs. (b-3) well-mixed PDMS (Sylgard 184, Dow Corning, MI, USA) pre-polymer components in a 10:1 ratio were degassed under vacuum until no bubbles appeared (20 $\sim$ 30 mins). A thin layer of PDMS was spin-coated on the SU-8 master to create cavities matching the LED shape. (b-4) After curing for 40 mins at 95 $^{\circ}\text{C}$, the PDMS substrate surface was treated with O₂ plasma to enhance adhesion between SU-8 and the PDMS surface. Then 800 μm SU-8 was spun on the plasma-treated PDMS substrate and (b-5) patterned with the backside exposure to form the microneedles. (b-6) After SU-8 development, the μ -waveguides array was polished by O₂ plasma. (b-7) After releasing the PDMS membrane from the glass wafer, (b-8) the device was assembled by polymeric bonding with shape-matching the PDMS cavities onto their corresponding LEDs.

4.3.3 A slanted μ -waveguides array fabrication and assembly

Fig. 4-3c depicts the core fabrication process flow of the slanted μ -waveguides array fabrication and assembly method. Specifically, (c-1) A 3" glass wafer was cleaned and dehydrated. A $\sim 50\ \mu\text{m}$ SU-8 layer was spun onto the wafer and patterned as the mock LEDs. (c-2) A thin layer of PDMS ($\sim 80\ \mu\text{m}$) was spun on the SU-8 master to form cavities matching the shape of the LEDs. (c-3) After the PDMS was cured at 95 $^{\circ}\text{C}$ for 40 mins, PR (S1813, Shipley, MA, USA) was patterned on the PDMS substrate to expose 7-mm-diameter circles, followed by O₂ plasma

treatment to convert the exposed lyophobic areas to the lyophilic ones. (c-4) After removing the PR mask, ~45 μL SU-8 3005 (MicroChem Corp., MA, USA) was dispensed on top of the plasma-treated PDMS surface using a micropipette. SU-8 droplets were pre-baked at 95°C for 12 hours in a vacuum oven, and (c-5) patterned with the backside exposure to form the microneedles followed by post-baking at 95°C for 30 mins. (c-6) After SU-8 development, the rough surface of the array was smoothed by O₂ plasma treatment at power of 300W and gas pressure of 0.5 Torr for 5 min and hard-baked at 150°C for 2 hours. (c-7) A thin layer of aluminum was thermally evaporated as a reflective shield for the μ -waveguides array. The metal layer on the tips then was removed using chemical wet etching, while the metal layer on the sidewall of the waveguide was protected using an SU-8 mask. The SU-8 mask was formed by dispensing SU-8 onto the region with the microneedles. During pre-baking, SU-8 reflowed due to the capillary effect, resulting in a thick resist layer surrounding the microneedles and a thin layer on the tip. After removing the thin SU-8 coating from the tip with oxygen plasma, the tip was exposed by metal wet etching, followed by dissolving the SU-8 mask in the developer. (c-8) The membrane carrying the microneedles array was released from the glass wafer, then (c-9) the cavities in the membrane were aligned to the LED chips on the μ -LEDs array and bonded with polymer adhesive.

4.4 Results

Fabricated prototype arrays and SEM images of the μ -waveguides are shown in Fig. 4-4. Several experiments were conducted to characterize the 3-D μ -waveguide arrays in three different aspects: optical, electrical, and mechanical properties.

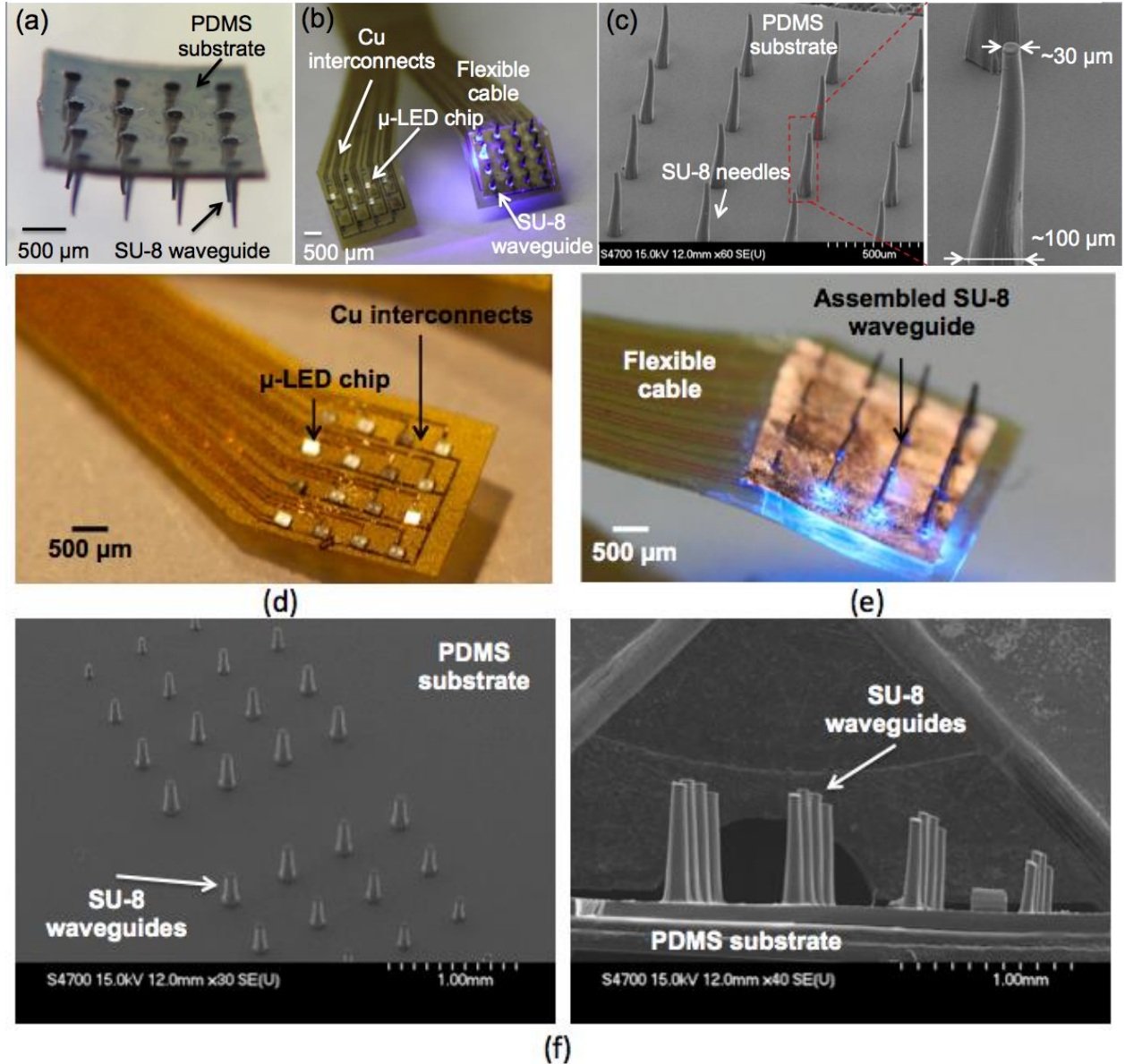


Figure 4-4. Fabricated prototypes of (a) a μ -waveguides array and (b) the assembled array with SU-8 waveguide. (c) SEM images of the SU-8 microneedle array fabricated on PDMS substrate. (d) before and (e) after assembly with a slanted microneedle array with the flexible LEDs array. (f) SEM images of slanted microneedle structures.

4.4.1 Optical properties

As important parameters for optical neural stimulation, the geometric effect of microneedles on the irradiance and total flux of transmitted blue light were characterized, using a ray- tracing method (TracePro®, Lambda Research Co., MA, USA). Normalized irradiance and total flux were measured at a 100-μm distance from the microneedle tip, as the function of the tip size (Fig.

4-5a). With a small tip size, optical throughputs tend to spread out, resulting in divergent irradiance, while a large tip size leads to concentrated irradiance with a confined output beam.

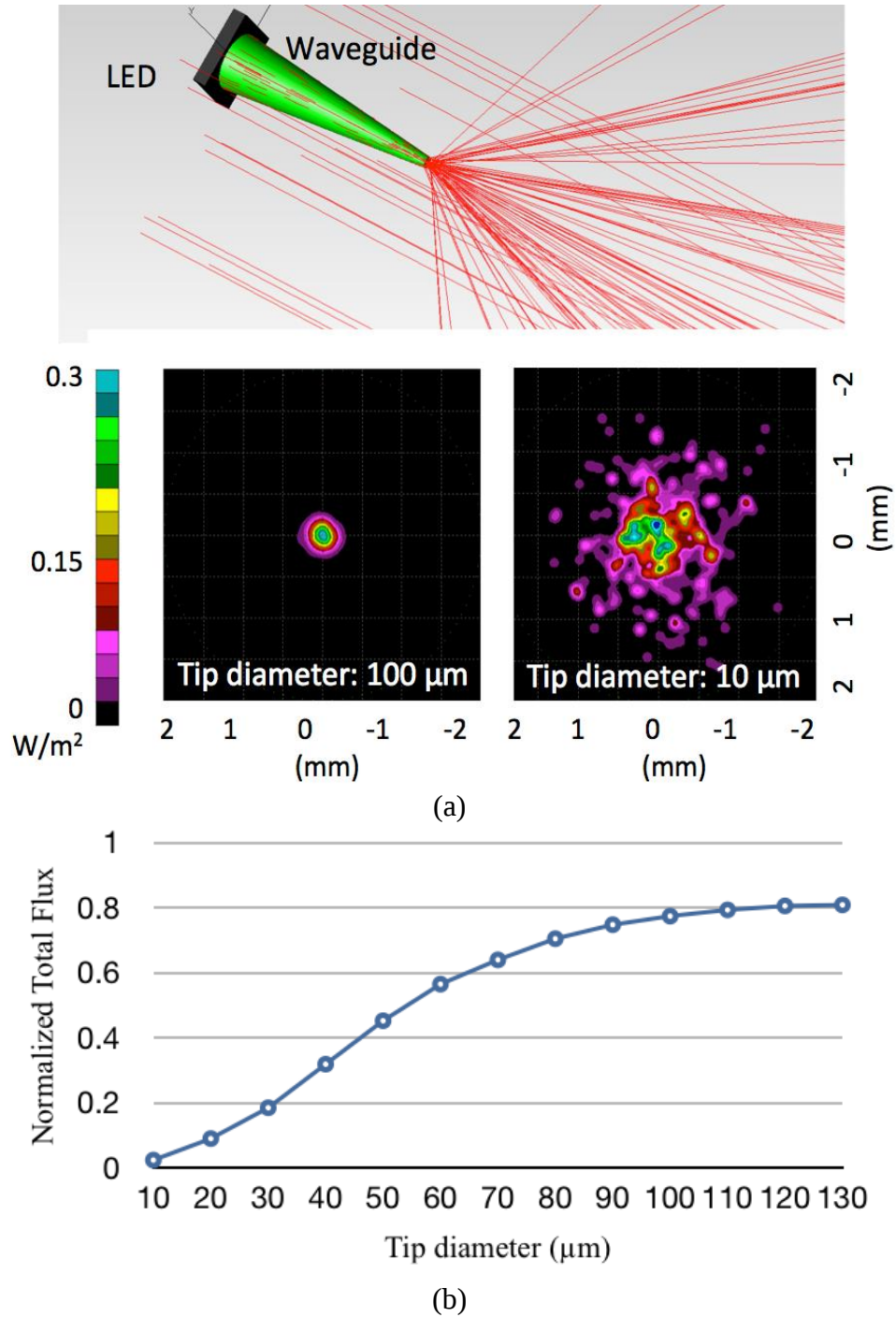


Figure 4-5. Simulation results: (a) irradiance measurements at a 100 μm distance from two tips with diameters of 10 μm and 100 μm , and (b) normalized total flux measured at a 100 μm distance from waveguide tips with different tip diameters (10 – 130 μm).

The coupling efficiency of the proposed slanted waveguide array was studied also. In this case, several assumptions were made such as a typical LED radiation angle of $\sim 60^\circ$ and inserted an 80- μm -thick PDMS layer (with the refractive index of 1.46 at 470 nm) between the LED and the SU-8 waveguide (with the refractive index of 1.59 at 470 nm). Maximum coupling efficiency of the waveguide with tip diameter larger than 100 μm was estimated to be around 80%, by assuming the sidewall of the waveguide was a perfect mirror and all incidental light from the LED entered the base of the waveguide. Simulation results suggest that the microneedle tip size must be designed carefully to deliver sufficient optical throughput to the target area, while permitting easy penetration of the tissue.

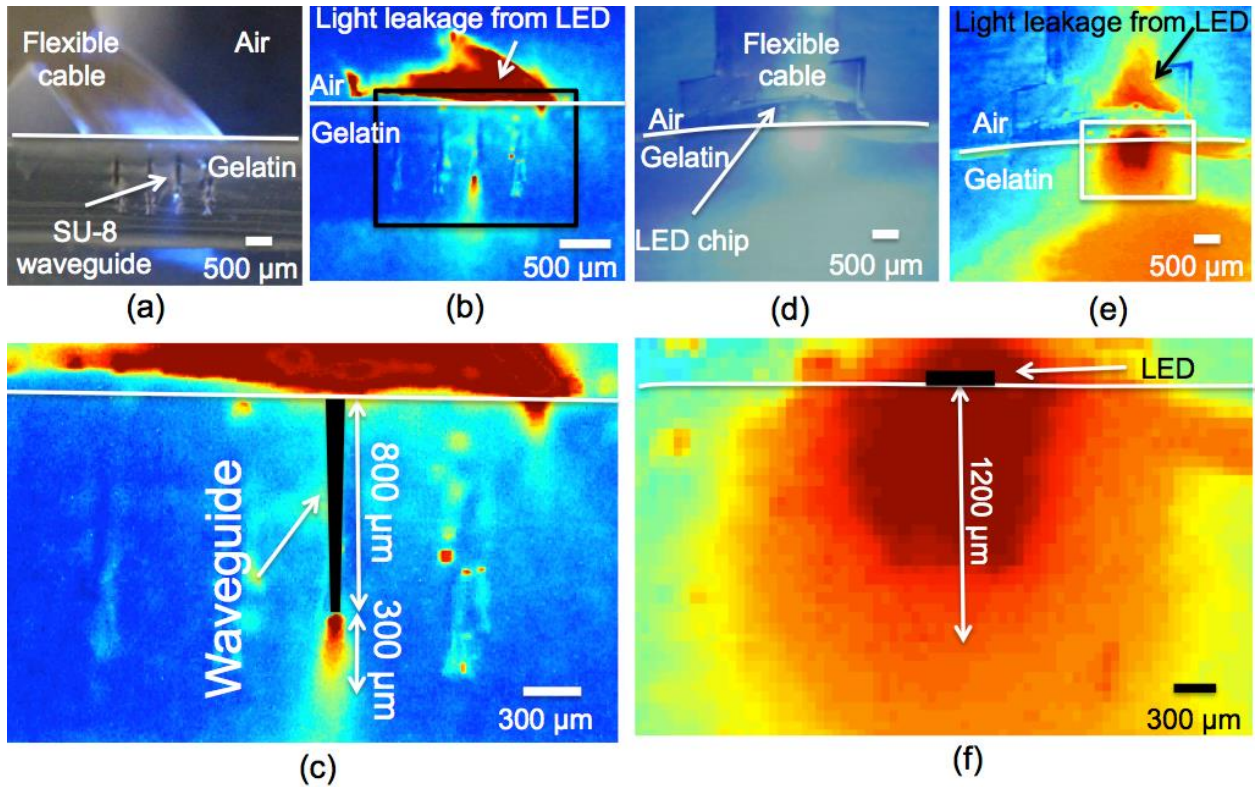


Figure 4-6. (a) Experimental setting to study optical throughput at the tip of waveguide, (b) relative intensity of the blue light (450-495 nm) extracted from the captured image, (c) the zoom-in view of the boxed area in (b). (d) Experimental setting to study optical throughput the μ -LED chip without the waveguide, (e) relative intensity of the blue light (450-495 nm) extracted from the captured image, (f) the zoom-in view of the boxed area in (e).

To characterize the light-scattering property of the array, the optical throughput at the tip of the μ -waveguide in a scattering media (20 % gelatin) was studied. A 3-D μ -waveguides array was placed on the gelatin media and images of the optical throughput at the tip of the single μ -waveguide were captured. The activated LED was driven by 2.7 V resulting in a power consumption of 3.4 mW, a temperature increase of 0.1 °C, and the light source irradiance of 1 mW/mm². Blue light spectra (wavelength range of 450 – 495 nm) were extracted from the captured image, and the normalized relative light intensity (maximum intensity as 1) was shown in Fig. 4-6b. A clear ellipsoidal scattering boundary ($\sim 600 \mu\text{m}$ in length and $\sim 100 \mu\text{m}$ in width) of the optical throughput, shown in Fig. 4-6b and c, indicated the waveguide effectively delivered the optical stimulation at the targeting depth without stimulating intermediate layers, in comparison with the large spherical scattering boundary of the μ -LED chip without the waveguide in Fig. 4-6f.

4.4.2 Electrical properties

A minimal irradiance of 1 mW/mm² must be delivered to the target area to induce action potentials. For surface stimulation, an input voltage of ~ 2.7 V was used to drive a single μ -LED chip to achieve the required optical power output, resulting in a power consumption of 3.4 mW. Considering typical coupling efficiency of the butt coupling (5 ~ 10 %) [131], the μ -LED chip was driven by an input voltage of ~ 2.9 V to achieve an optical power intensity of 10 mW/mm², resulting in a total power consumption of 17 mW and a temperature increase of ~ 0.4 °C.

4.4.3 Mechanical properties

Mechanical reliability of the μ -waveguides array was examined by a penetrating test. The μ -waveguides array was inserted into and retracted from the 20% gelatin media 10 times, and cracks or breakages on the μ -waveguide were examined microscopically. Among 10 samples, no

visible damage was observed, indicating mechanical rigidity of the μ -waveguide. The result suggested possible array implantation with intact dura, which helps minimizing additional complications caused by the dura-removal procedure.

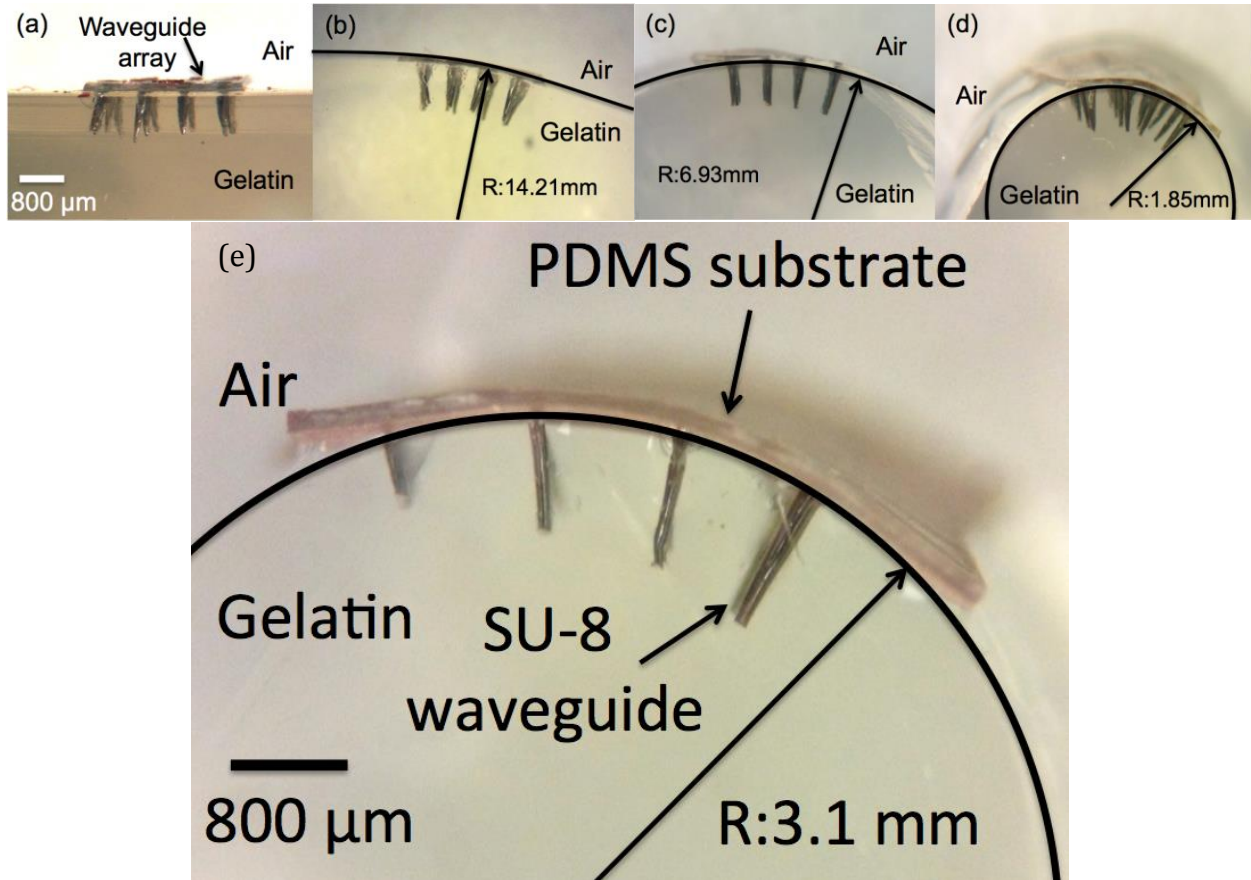


Figure 4-7. Penetration profile of (a-d) the 3-D μ -waveguides array and (e) the slanted μ -waveguides array in different curvatures of gelatin surface.

Flexibility of this array was also examined by implanting it in gelatin (20% w/w) with different surface curvatures. As shown in Fig. 4-7, the flexible PDMS substrate wrapped around the curved surface conformally, while the waveguides penetrated perpendicular into the bulk gelatin. This result demonstrated that a combination of the flexible substrate and the rigid waveguide structure offers potential applications for not only cortical implants, but also spinal cord and peripheral nerves implants. Specifically for peripheral nerve implants, each row of

waveguides can target different depths of axon bundles in the peripheral nervous system without deforming nerves.

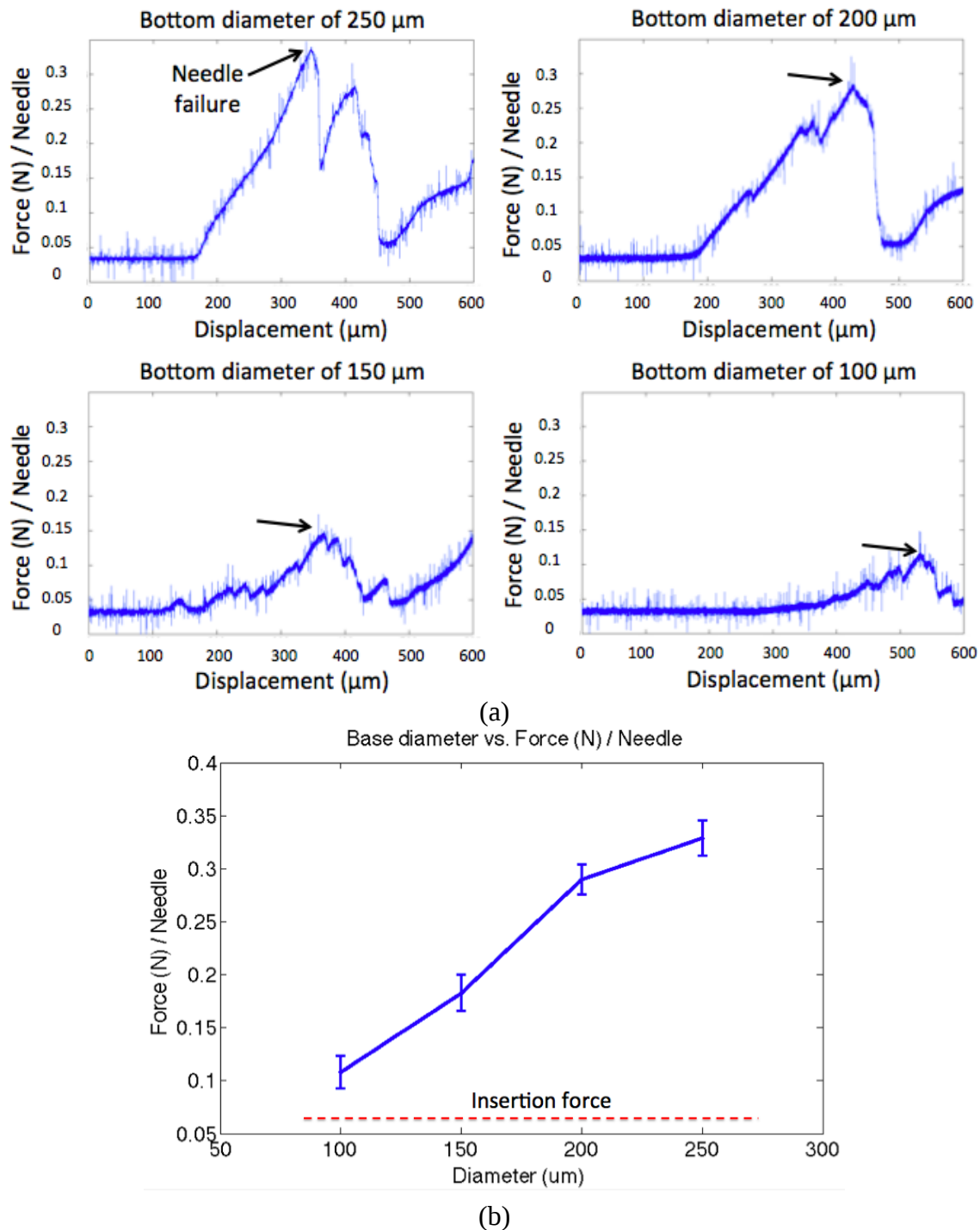


Figure 4-8. Evaluation of mechanical robustness of microneedle arrays using the axial loading test: (a) from the top left, force measurement vs. displacement of the microneedle array with different base diameters of 250, 200, 100, and 150 μm . (b) Needle failure force vs. base diameter.

Also studied was the mechanical failure of microneedles with different base diameters, based on the axial loading test described in [133], using arrays of four single-length microneedles. These had various base diameters of 100, 150, 200, and 250 μm , constant length of 900 μm , and tip diameter 25 μm . Previous study has shown that an interfacial needle area at the tip plays an important role with respect to insertion of a microneedle into skin, while other geometric parameters are less important [134]. According to this study, a 0.058 N/needle insertion force would be required for a microneedle with tip diameter 25 μm , corresponding to the interfacial area of 490 μm^2 [134].

During the test, each array was placed on a force gauge (Mark-10 BG5, Mark-10 Co., New York, USA) attached to a syringe pump (HA2000P, Harvard Apparatus, MA, USA). The station pressed the microneedle array against a rigid glass surface at constant speed of 0.1 mm/s. Displacement of the station measured from a laser distance sensor (OADM 20I6441/S14F, Baumer Electric, Germany) and the corresponding force measured via the force gauge were acquired using a dSPACE system (DS1104, dSPACE Inc., Germany).

Maximum force measured immediately before a reduction was seen as the critical force required for needle failure due to buckling. Fig. 4-8a shows the failure forces for individual microneedles with four different base diameters, estimated by dividing the total measured force by the number of microneedles on each array. Ten sample arrays were measured for each base diameter; the relationship between the failure force and the base diameter is shown in Fig. 4-8b (the error bar indicating standard deviation). Results suggest that microneedles with base diameters larger than 100 μm are mechanically robust enough to facilitate penetration into skin, since average force of the microneedle with a 100 μm base diameter (0.12 N/needle) is above the required insertion force (0.058 N/needle) in [134].

4.4.4 Long-term biocompatibility

Last, histological analysis was performed to evaluate long-term biocompatibility of the SU-8 microneedles, and to identify any evidence of trauma associated with the implanted device. All animal surgical and postoperative care procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University.

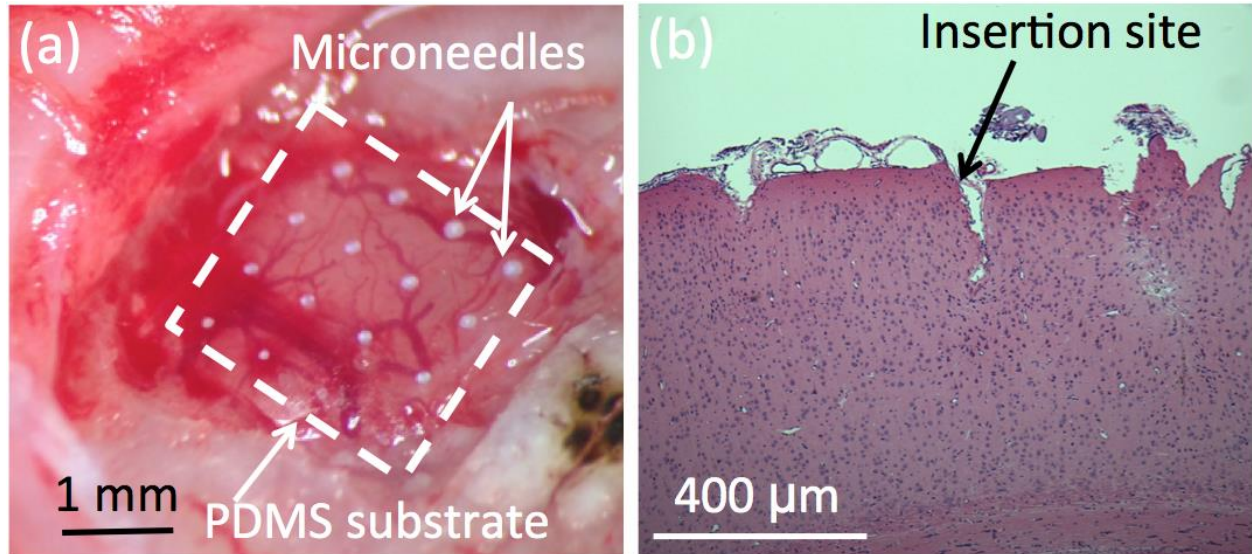


Figure 4-9. Histological analysis for long-term biocompatibility evaluation: (a) 4×4 slanted microneedle array was implanted on rat V1. (b) A coronal section ($5 \mu\text{m}$ -thick and stained with H&E) of the V1 after 6 months implant.

For device implantation, three rodent subjects (Sprague-Dawley rats: 250 – 400 g) were anesthetized and placed in a stereotaxic apparatus. Using sterile surgical procedures, a 3–4 cm incision was made in the skin overlying the skull and a small region of bone was removed to expose primary visual cortex (V1). Dura mater was carefully removed, then a 4×4 slanted waveguides array (300 – 1200 μm in length and $\sim 100 \mu\text{m}$ in tip diameter) was implanted in V1, as shown in Fig. 4-9a. Gel-form was applied on the craniotomy and the skin overlying the skull was sutured closed. The animals were allowed to recover from anesthesia, housed separately, and given pain medication and antibiotics to minimize infection. After recovery from surgery, the rats were observed daily to detect any abnormal behavior due to the implant. None of the three

rats were used in the preliminary study showed noticeable behavioral changes. They then were euthanized at 1-, 3-, and 6-month periods, and histological analysis was performed. After probes were implanted, the brains were dehydrated in graded concentrations of ethanol, and embedded in paraffin. The implanted hemisphere was sectioned into 5 μm coronal sections and stained with hematoxylin and eosin (H&E). From Fig. 4-9b, mild glia cell formations and a small region of tissue compression were observed around the microneedle tracks. This result indicated that the flexible array combining a PDMS substrate with SU-8 microneedles would not cause significant damage to the brain tissue, thus is suitable for long-term cortical implantation.

4.5 Conclusion

Design objectives, fabrication process, and the properties of 3-D μ -waveguide arrays for optogenetics are described in this chapter. The 3-D μ -waveguide was designed to target layer IV in V1 of the rat, and the fabrication was based on SU-8 μ -needle fabrication using the backside exposure technique. Slanted μ -waveguides array was designed with lengths ranging 400 to 1000 μm to provide access to multiple cortical layers using a new fabrication method (DBE) for slanted microneedles fabrication.

Comprehensive experiments studied the mechanical, optical, and biocompatible properties of the as-fabricated arrays. The simulation result of optical throughput at the tip of the waveguide was demonstrated and its scattering characteristic was analyzed. The presented image-processing method was suitable for analyzing relative intensity distribution of the optical throughput. Electrical power consumption to achieve irradiance of 1 mW/mm^2 at the waveguide tip was estimated based on 10% coupling efficiency of the butt coupling. Mechanical failure and *in vivo* animal tests validated that slanted microneedle arrays are mechanically robust and biocompatible, suitable for chronic neural implants. Mechanical properties of the array were

demonstrated via the penetration test on gelatin media, and results suggested possible applications of spinal cord and peripheral nerve implant.

CHAPTER 5

Varying-length polymer microneedle arrays fabricated by droplet backside exposure

In the previous chapter, the slanted μ -waveguides array was introduced as an optical neural interface for applications in optogenetics-based neural stimulation. A new fabrication method was developed for making slanted microneedle structures monolithically on a flexible polymer substrate, and this chapter presents a single-step backside ultraviolet (UV) lithography method, namely droplet backside exposure (DBE). To demonstrate the feasibility of the DBE approach, SU-8 microneedle arrays were fabricated on PDMS substrates. Microneedle length was controlled by tuning the volume of the SU-8 droplet, utilizing the wetting barrier phenomenon at a liquid-vapor-hydrophilic surface-hydrophobic surface interface. Experimental results supported excellent repeatability and controllability of the DBE method for microneedle fabrication. Analytical models and finite element method (FEM) were studied to predict microneedle dimensions, which agreed with the experimental data. To verify versatility of the proposed DBE method, a slanted hollow microneedles array was implemented.

5.1 Motivation

Microneedle based on microelectromechanical systems (MEMS) technologies have been an attractive topic for decades, since they can serve as an interface between an organic system and an external micro device [135]. Such interfaces are capable of either delivering a liquid substance such as drug, protein, and nano-particles into the organism, or extracting biofluids from the organism for analysis [136]. In addition, microneedles can be used as microelectrodes

to interface with the nervous system for neural recording and stimulation [137]. Recently, optically-transparent microneedles made of glass [25] and polymer [30] were reported as μ -waveguide for optical neuromodulation based on optogenetics, which extend the potential of microneedles.

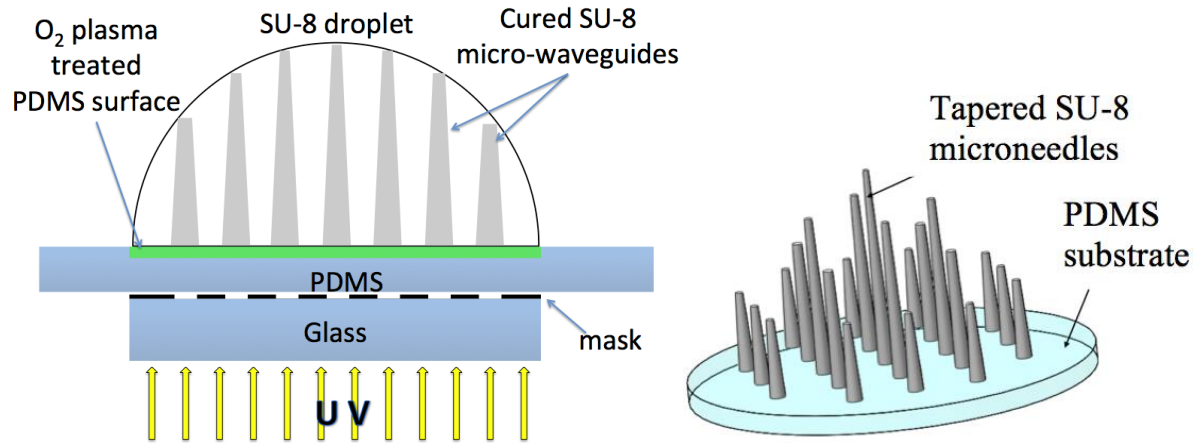


Figure 5-1. Principle of the proposed droplet backside exposure (DBE) method.

Depending on their geometries, typically, microneedles can be classified into two groups: in-plane microneedles and out-of-plane microneedles. In most cases, in-plane microneedles are fabricated with conventional silicon MEMS techniques, and can be used for precise drug delivery to a target region or tissue of the body [135]. Contrarily, out-of-plane microneedles require complex fabrication processes, and can be made over a large area and with a high yield and excellent repeatability. Out-of-plane microneedles have been used widely in hypodermic drug delivery applications [138] and cortical neural interface [1]. Accordingly, this author focuses discussion on microfabrication techniques for making out-of-plane microneedles.

To date, a wide variety of materials, including silicon, metal, glass, polymers, and biodegradable materials, have been used to fabricate out-of-plane microneedles, depending on a target organism and/or a specific application of the interface [136]. Various microfabrication methods also have been developed based on different building materials. For example, silicon-

based out-of-plane microneedles typically were fabricated using either reactive-ion etching (RIE) [136] or a combination of anisotropic wet etching, deep-RIE, and anisotropic bulk micromachining processes [139], [140]. Metal microneedles were developed in [141], using electroplating onto a seed layer sputtered on polymer or silicon micromolds. Moreover, glass microneedles were made using a conventional micropipette puller, or micromachining then etching a thick glass substrate [25]. However, these fabrication approaches are labor intensive and costly. With superior biocompatibility and low-cost manufacturing capability, polymeric materials have become ideal material candidates for constructing microneedles [129], [133], [138]. Previously- reported methods include soft lithographic replication by melting polymer into a master mold, as well as backside exposure using photoresist such as PMMA and SU-8 [129], [138].

Despite advances in microfabrication, microneedle arrays fabricated with the aforementioned methods typically have a uniform length, primarily designed for applications of hypodermic drug delivery. These arrays are unsuitable for specific applications requiring comprehensive access to different locations of an organism, where naturally have distinctive functionalities. As an example, to restore motor and sensory functions, peripheral neuroprostheses interfacing with peripheral nerves or muscles require selective stimulation of a large number of individual muscle groups with graded recruitment of force. In addition to peripheral neural interfaces, BMIs utilize the cerebral cortex as a major target because of its accessibility and its key role in cognitive, sensory perception, and motor control [1]. A distinguishing feature of the cerebral cortex is a layered structure, where different cortical layers contain a characteristic distribution of neuronal subtypes and connections with other cortical and subcortical regions. For these applications in neuroprosthetic interfaces, a varying-length or

slanted microneedle array is necessary. One such example is the Utah Slanted Electrode Array (USEA), which has been demonstrated to provide comprehensive access to multiple independent motor neuron subpopulations [142]. While there is a critical need for slanted microneedle arrays, fabrication techniques for making such arrays have not been studied extensively. Currently, only some methods have been reported. In particular, the USEA was fabricated by dicing and etching bulk materials, such as silicon or glass [25], [137]. However, this method relies on specialized micromachining tools and bulk materials, which may limit its adaptation by other researchers in this field. Stacking in-plane microneedles with different lengths is another way to build a varying-length microneedle array, but this approach requires a complex assembly process [133]. While polymer-based backside exposure method allows for precise length control with different mask aperture sizes [143], independent control of microneedle dimensions (length, tip and bottom diameters) is not possible.

To address these challenges, a unique fabrication technique, called droplet backside exposure (DBE), has been proposed for making polymer-based (SU-8) varying-length microneedle structures. Microneedles with different lengths can be achieved using the height variance in the profile of a droplet structure through rapid, single-step UV lithography (Fig. 5-1). This DBE technique enables the independent control of the length, tip and bottom diameters of individual microneedles, without using a specialized machine or complex fabrication techniques. The following sections will detail the principle of the proposed method, the DBE fabrication steps, and its adapted processes for making slanted hollow microneedle array. Experimental results have demonstrated the repeatability and versatility of the proposed fabrication method.

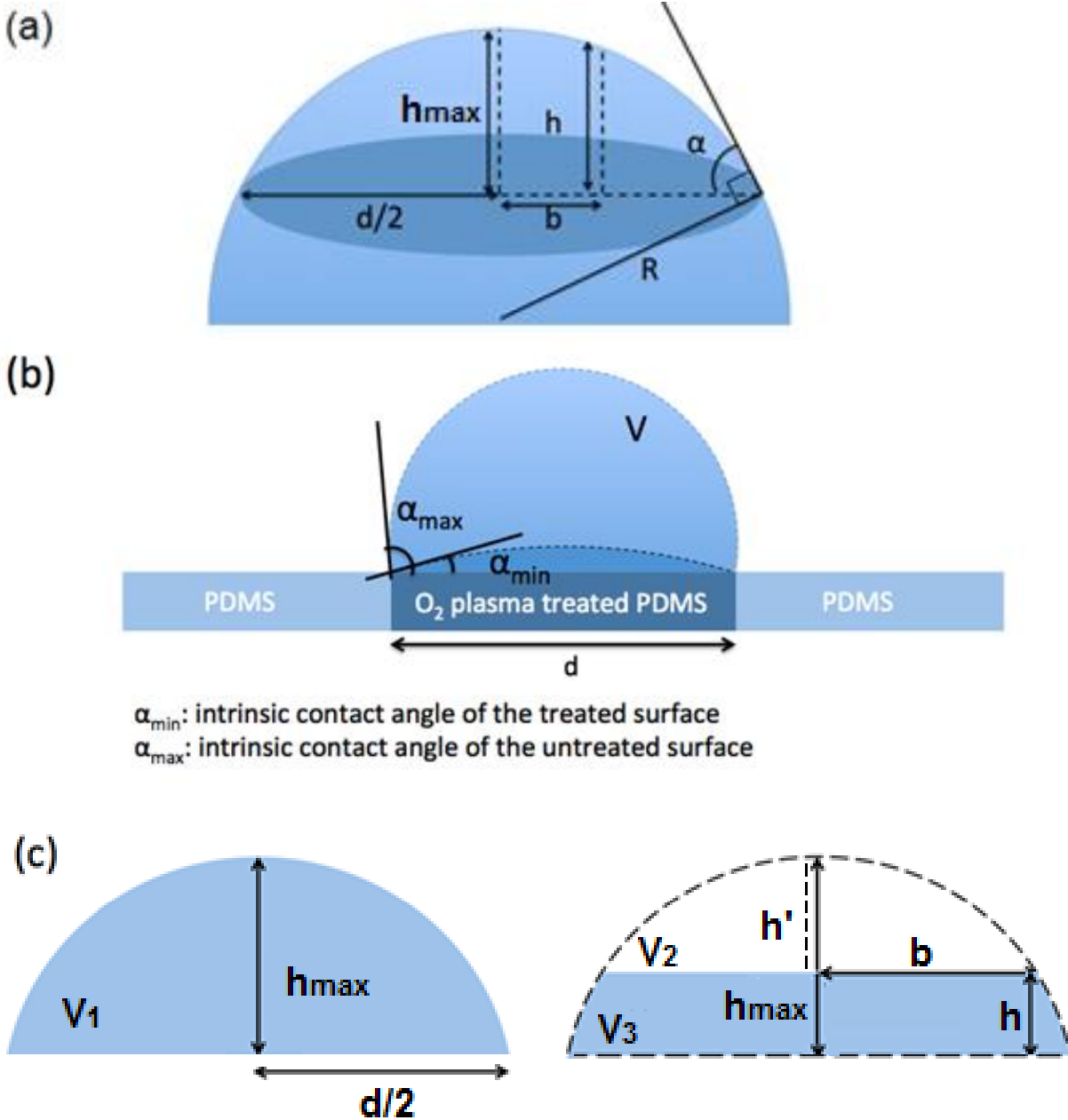


Figure 5-2. An analytical model for designing slanted microneedle arrays, approximated based on the geometry of an ideal partial sphere droplet: (a) a diagram of an ideal partial sphere, or a spherical cap; (b) the ideal droplets, formed on the hydrophilic surface, with a minimum CA ($\alpha_{\min}$) and a maximum CA ($\alpha_{\max}$), respectively; (c) the relationship between two partial spheres, V_1 and V_2 .

5.2 Methods

5.2.1 Principle of the DBE

The proposed DBE technique takes advantage of a wetting barrier phenomenon occurring at a four-phase interface [144]. Generally, the shape of a liquid droplet on a homogenous solid substrate is primarily governed by the surface free energy of the substrate. However, for a four-phase interface such as a vapor-liquid-hydrophilic surface-hydrophobic surface interface, the spreading of the liquid is restricted by the wetting barrier formed at the boundary between the hydrophilic and hydrophobic regions. Consequently, the contact angle (CA) of the droplet can vary within a certain range, with a maximum value corresponding to the equilibrium CA of the liquid on the hydrophobic surface, as shown in Fig. 5-2b.

If it is assumed that the droplet is a perfect partial sphere with a pre-defined diameter, a relationship between the CA and the volume of the droplet can be estimated. Based on this principle, an analytical model was derived to determine the maximum volume of the liquid sustained in a pre-defined pattern and to predict the length variation of microneedles. At equilibrium, the volume of the liquid can be calculated using the following equation:

$$V = \frac{1}{3}\pi R^3(2 - 3\sin(90 - \alpha) + \sin^3(90 - \alpha)) \quad (1)$$

where V is the actual volume of the liquid delivered to the substrate and α is the equilibrium CA of the droplet. The diameter of the interface between the droplet and the hydrophilic surface, denoted as d in Fig. 5-2b, is pre-defined so the relationship between R and d can be derived as:

$$R = \frac{d}{2\cos(90-\alpha)} \quad (2)$$

By substituting R with d in Eqn.1, the liquid volume can be rewritten as a function of the pre-defined interfacial diameter and the equilibrium CA (α) of droplet.

$$V = \frac{1}{24} \pi d^3 \frac{(2+\cos\alpha)(1-\cos\alpha)^2}{\sin^3\alpha} \quad (3)$$

Once the CA of the droplet is determined by the volume, the profile of the droplet can be estimated. $h_{\max}$ is defined as the maximum height of the droplet, which can be estimated using a trigonometric relationship of R and α shown in Fig. 5-2a.

$$h_{\max} = R - \frac{d}{2} \tan(90 - \alpha) = R - \frac{d}{2 \tan \alpha} \quad (4)$$

Then the length (h) of a microneedle formed at a certain distance (b) from the center of the droplet can be calculated using a relationship between two partial spheres V1 and V2 in Fig. 5-2c, as given below:

$$h = \frac{\sqrt{\left(\frac{d}{2}\right)^4 + h_{\max}^2 \left(2\left(\frac{d}{2}\right)^2 - 4b^2 + 1\right) - \left(\frac{d}{2}\right)^2 + h_{\max}^2}}{2h_{\max}} \quad (5)$$

In this study, the choice of liquid was SU-8 for polymer-based microneedle fabrication. Particularly, SU-8 3005 (with 50% resin solid, MicroChem Corp., MA, USA) was used, because its low viscosity (65 cSt) allows accurate volume dispense. Polydimethylsiloxane (PDMS) was selected as the flexible substrate material, because the innate PDMS surface is hydrophobic to the primary solvent of SU-8, cyclopentanone, and effectively can be modified to super hydrophilic through an oxygen (O₂) plasma treatment. Among a same array, the dimensions of a microneedle, including its tip diameter, base diameter, and length, can be designed precisely based on the above analytical model. More specifically, base diameter is determined by the

diameter of mask aperture, while tip size is associated closely with the inclined angle of the tapered microneedle and the thickness of the SU-8 coating [143]. Since the inclined angle is mainly controlled by the separation between the absorber on the photomask and the top surface of the SU-8 droplet [129], the only adjustable parameter is the thickness of the SU-8 layer. Because this thickness varies in a predictable manner across the SU-8 droplet, the length and tip diameter can be controlled by tuning the distance from the mask aperture to the center of the droplet.

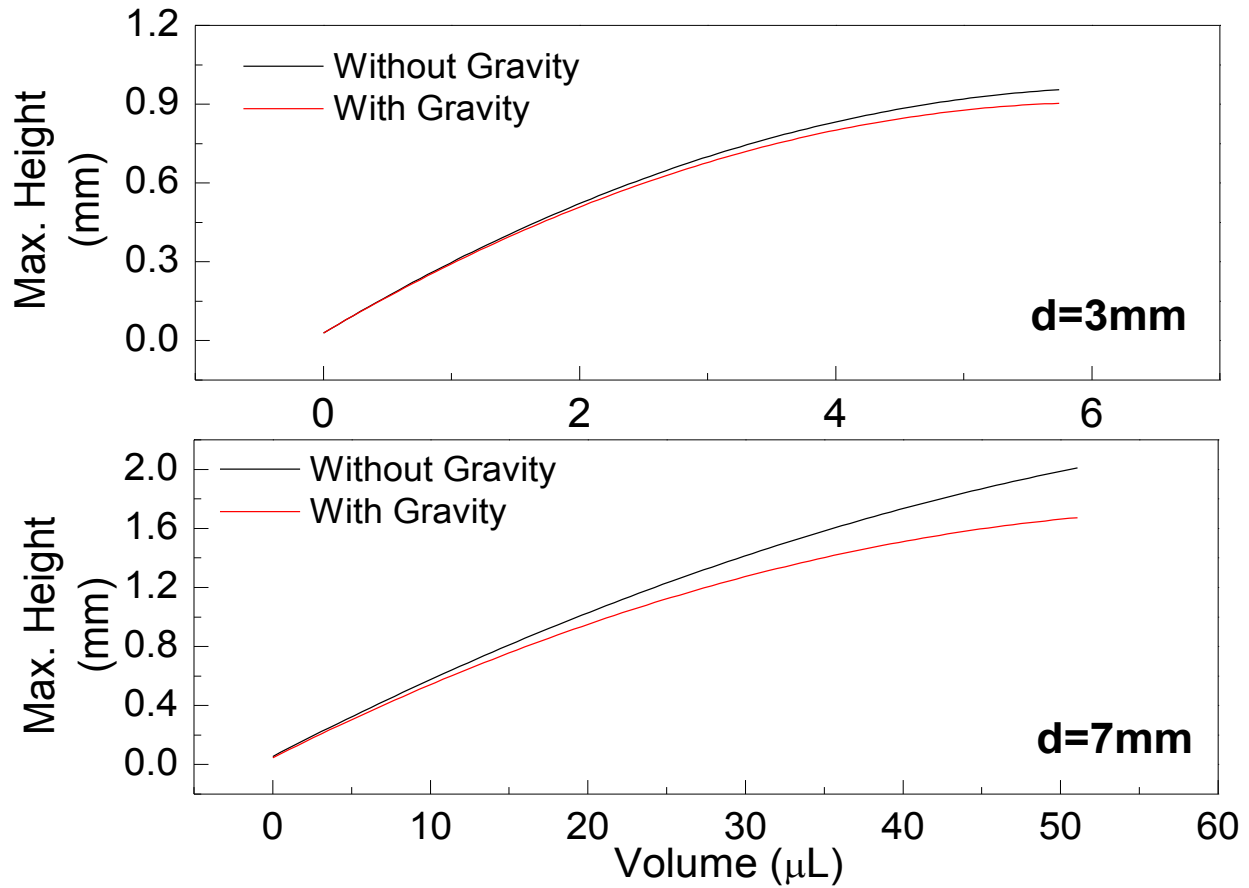


Figure 5-3. Finite element stimulation result of gravity effect on the maximum height of SU-8 droplets with different base diameters.

5.2.2 Volume shrinkage and gravity effect

Factors that must be considered to achieve more accurate length estimation: the gravity effect of the droplet and the volume shrinkage caused by solvent evaporation and cross-linkage. When a droplet is placed on a horizontal surface, the droplet shape will be deformed by the gravity force. This effect depends on the size of the droplet as well as the given materials. Since the proposed method assumed that the droplet is an ideal partial sphere, the analytical estimation would not be accurate for a large-size droplet with significant gravity effect. Vafaei et al. reported an analytical model to estimate the droplet geometry under gravity effect in [145], but this model requires experimental measurements of the maximum height, CA radius, and equilibrium CA of the droplet.

To study the gravity effect, finite element simulation in COMSOL Multiphysics 4.3 (COMSOL, Inc., MA, USA) was performed using the Laminar Two-Phase Flow (Level Set) model. Two different diameters of the plasma-treated hydrophilic circle (d), 3 mm and 7 mm, were studied. The equilibrium CAs of SU-8 was about $\sim 10^\circ$ on the plasma treated PDMS and $\sim 58.7^\circ$ on the untreated PDMS, measured by a CA analyzer. The simulation used $7.95 \text{ mm}^2/\text{s}$ for the kinematic viscosity of SU-8 3005 at an elevated temperature (between 60°C and 95°C) [146]. Two simulation parameters were assumed: the SU-8 density of 1.075 g/cm^3 and the surface tension of $\sim 48 \text{ mN/m}$ [146]-[148]. The droplet was defined as a hemisphere at $t = 0 \text{ sec}$, with a radius varied from 0 mm to 1.4 mm in the case of a 3 mm base diameter and from 0 mm to 3.4 mm in the case of a 7 mm base diameter, in $10 \mu\text{m}$ increments. During the simulation, the droplet continuously spread over the heterogeneous substrate until its CA reached the maximum equilibrium CA ($\sim 56^\circ$ for SU-8 on untreated PDMS). Then the maximum height of the droplet for each individual radius was recorded before and after applying the gravity effect, and plotted

as a function of the droplet volume (Fig. 5-3). Simulation results suggest that the gravity effect on the maximum height of the droplet was negligible when droplets had a small base diameter and low volume sizes. For a large base diameter of 7 mm, when the droplet volume was greater than 25 μL , the gravity effect caused a significant reduction in the maximum height by over 10 %. Compared to the experimental data, the current analytical model is considered valid with an error of smaller than $\sim 8.0\%$, for a droplet with a base diameter of ≤ 7 mm and a volume of ≤ 25 μL . Slight oscillation of the SU-8 surface was observed during the transient analysis of the Level Set model, which was assumed to have a negligible effect on the droplet shape.

Volume shrinkage due to solvent evaporation and SU-8 cross-linking was modeled by assuming that the droplet still remains a spherical shape after pre- and post- baking. The volume shrinkage factor of $\sim 7.5\%$ was estimated based on empirical analyses where the contact angles and the volumes of one hundred droplets were measured and compared before and after baking. The empirical result is consistent with the findings reported by Lorenz et al. [147]. Applying this factor in Eqn. 1 results in a new formula for calculating the actual droplet volume, V_{act} , as expressed in Eqn. 6.

$$V_{\text{act}} = V \times S = \frac{1}{24} \pi d^3 \frac{(2+\cos\alpha)(1-\cos\alpha)^2}{\sin^3\alpha} \times S \quad (6)$$

where S is a percentage of solid in SU-8 solution multiplied by 0.925.

5.3 Fabrication methods

5.3.1 Basic DBE fabrication process

Fig. 5-4a depicts the core fabrication process flow of the DBE method. Specifically, (a-1) PDMS pre-polymer components (Sylgard 184, Dow Corning, Midland, MI) were well mixed in a 10:1 ratio, and the mixture was degassed under vacuum until no bubbles appeared (20 ~ 30 min).

PDMS was then spun on a clean 3" glass wafer. After the PDMS was cured for 40 min at 95 °C, photoresist (PR, S1813, Shipley, Marlborough, MA) was spun on the PDMS.

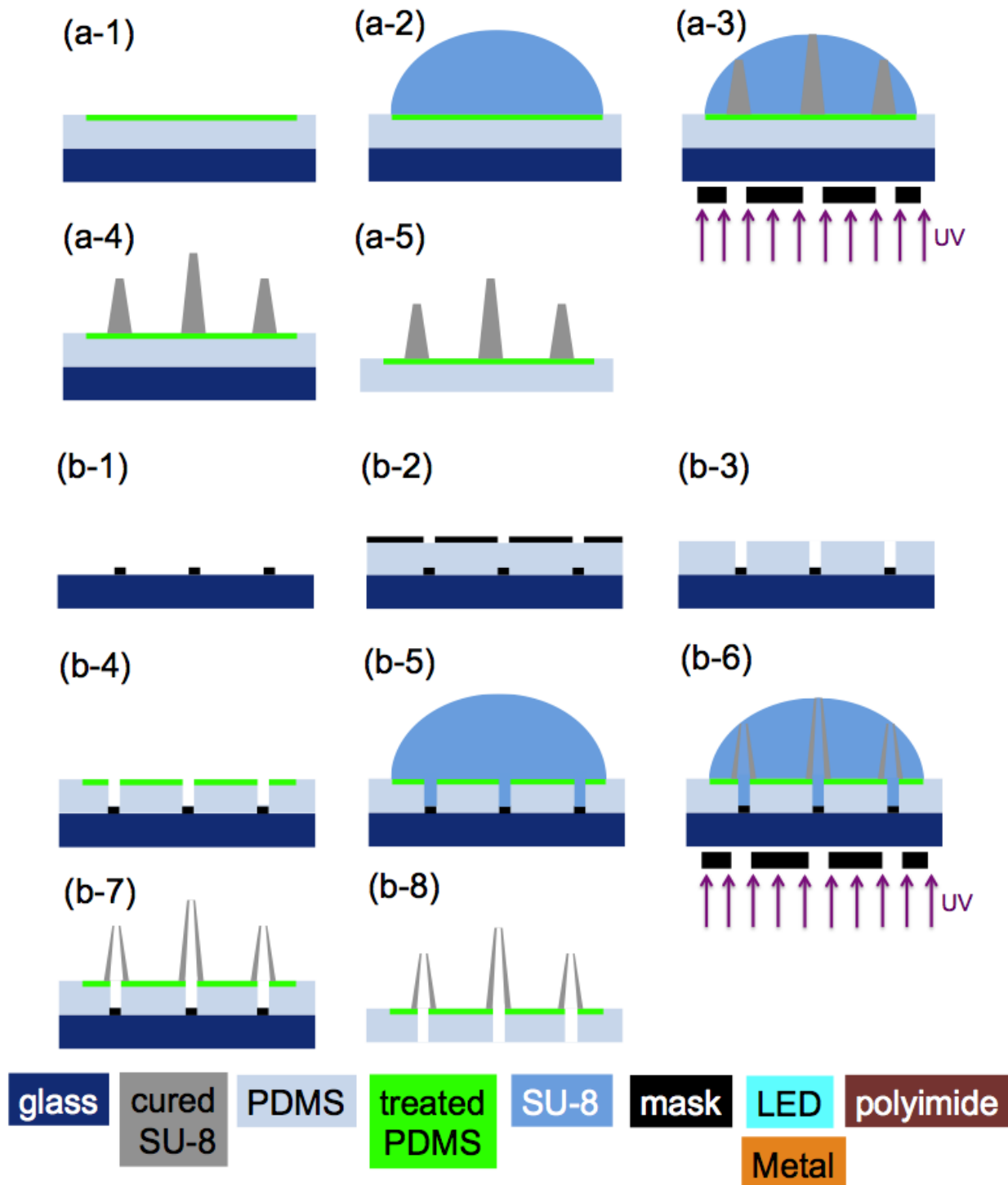


Figure 5-4. The core fabrication process of the DBE method and its variation: (a) the core DBE fabrication method; (b) a slanted hollow microneedle fabrication process flow.

Circular patterns with various diameters (3 ~ 7 mm) were formed in the PR layer through photolithography, followed by an O₂ plasma treatment to define hydrophilic areas for containing SU-8 droplets. (a-2) After removing the PR masking layer, SU-8 3005 was dispensed on top of the plasma treated PDMS areas using a micropipette. Then SU-8 droplets were pre-baked at 95 °C for 12 hours in a vacuum oven, and then (a-3) patterned with the backside exposure to form slanted microneedle arrays. (a-4) After SU-8 development, the slanted microneedle arrays were polished with O₂ plasma etching. (a-5) Finally, the flexible microneedle arrays were released from the glass wafer.

5.3.2 A slanted hollow microneedle array fabrication process

The basic DBE method is fully adaptable to the fabrication of hollow microneedles, by constructing a multi-layer masking structure for the backside exposure process. In this case, a metal mask was first patterned on the top side of a glass wafer, then a second mask was placed on the bottom side of the same wafer after pre-baking the SU-8 droplet. Due to the different separations between the two masks and the bottom of the SU-8 droplet, the outside wall of the microneedle will have a larger inclining angle, while the inside wall will have a smaller angle, resulting in a hollow needle structure [129].

Fig. 5-4b depicts the fabrication process flow for making slanted hollow microneedles using the modified DBE method. Overall fabrication flow was similar to the core DBE process introduced in Fig. 5-4a. Additionally, two important steps were incorporated, to form channels through the PDMS substrate and the microneedles. Briefly, (b-1) a 3” glass wafer was cleaned by IPA, acetone, and deionized (DI) water, then a metal (Al) mask was patterned on the wafer. (b-2) Approximately 80- μ m-thick PDMS was spun (800 rpm for 40 sec) and cured for 40 min at 95 °C. Then another layer of metal was deposited and patterned as a RIE etching mask. (b-3) A mi-

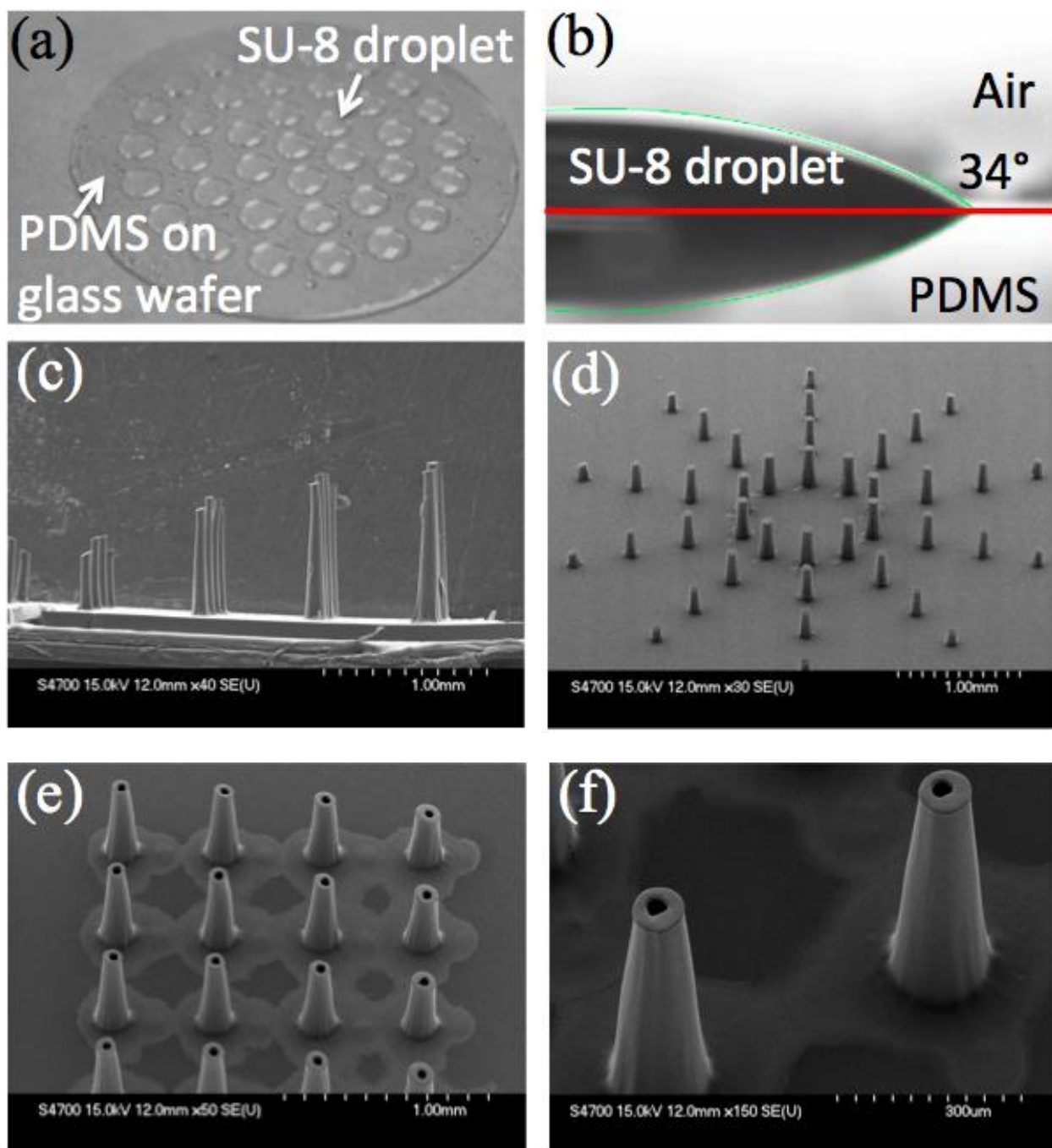


Figure 5-5. (a) Dispensed SU-8 droplets on PDMS. (b) Example of CA measurement. SEM images of (c) the inclining profile of the microneedles with a 35.2° CA, and (d) the aerial view of a circular microneedle array fabricated using a 20 μ L droplet. (e) and (f) SEM images of a hollow microneedle array.

xture of CF₄ and O₂ was utilized as the reactive gas to etch through the channels in the PDMS layer. (b-4) After removing the RIE etching mask, the subsequent processing steps were the same as the steps (a-2) to (a-5) in the core fabrication process.

5.4 Results

Prior to microneedle fabrication, the equilibrium CAs of the SU-8 on both intact and plasma-treated PDMS substrates were measured using a CA analyzer, as shown in Fig. 5-5b, to guide the design of microneedle arrays. The intrinsic CAs on hydrophobic, $\alpha_{\max}$, and hydrophilic, $\alpha_{\min}$, PDMS substrates were $\sim 58.7^\circ$ and $\sim 10^\circ$, respectively. With an interfacial diameter (d) of 7 mm, the maximum height of the droplets ($h_{\max}$) was estimated to range from 306 μm to 1968 μm , corresponding to the droplet volumes of 18 μL to 38.7 μL .

Fig. 5-5c shows a slanted microneedle array, which was fabricated with the droplet diameter of 7 mm using the proposed DBE method. This array had an overall slope of $\sim 35.2^\circ$, resulted from the droplet volume of 20.4 μL . Circularly arranged microneedles, fabricated from a 20 μL droplet, are shown in Fig. 5-5d. The SEM images of the as-fabricated hollow microneedle array were given in Fig. 5-5e and f.

Consistency of the DBE method was first investigated empirically with a fixed droplet volume. In this study, ten 25 μL SU-8 droplets were dispensed on O₂ plasma treated PDMS circles 7 mm in diameter, resulting in an average CA of 41.5° . Ten waveguide arrays were fabricated from these droplets using the basic DBE method as described previously. For the backside exposure, it has been found that the tip size of the tapered microneedles increases as the separation between the mask and the top SU-8 layer decreases [129].

To achieve a uniform tip size of microneedles, the aperture diameter of the mask was changed from 140, 120, 100, to 80 μm , corresponding to the distance from the droplet center of

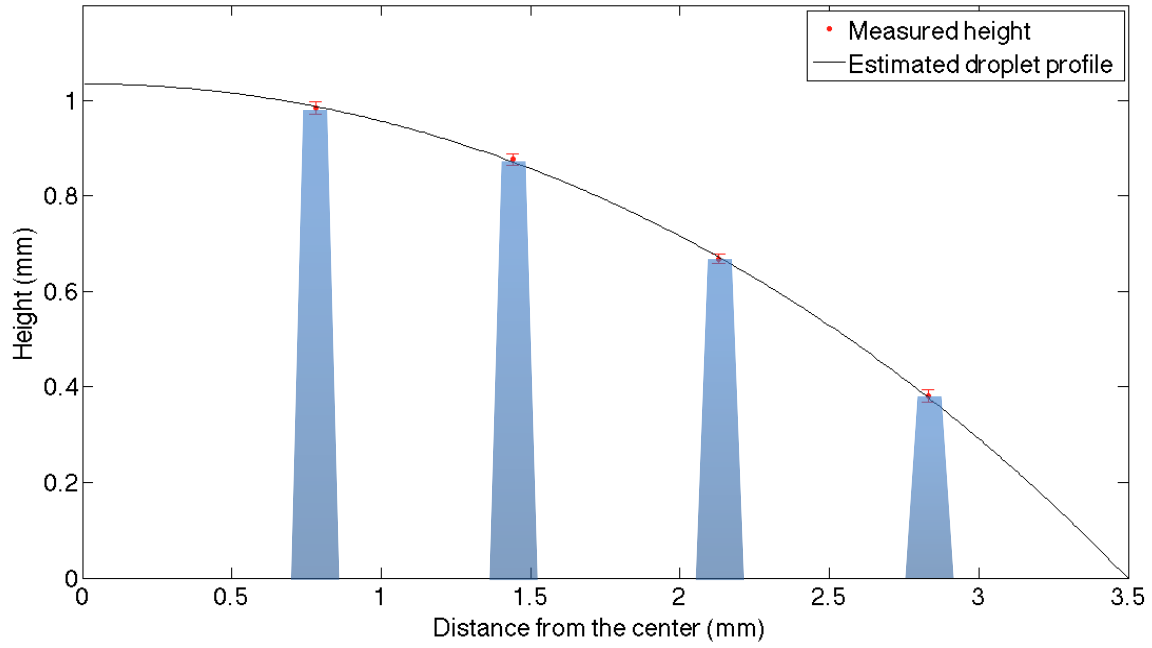
780, 1440, 2130, and 2830 μm . Average heights of fabricated microneedles were measured and plotted in Fig. 5-6a, depending on the estimated droplet profile. Waveguide lengths were close to estimated values with standard deviations of less than 5%, demonstrating the excellent repeatability and reliability of the proposed DBE method.

Further, the relationship between droplet volume and microneedle length was studied. With the same base diameter of 7 mm, the droplet volume varied from 10 to 27 μL with a 0.5 μL increment, and the same mask apertures described above were used. Ten arrays of microneedles were made for each volume size, and their lengths were measured from the SEM images and compared with the theoretical values estimated from Eqn. 6. The measured heights of the microneedles fitted well with their estimated lengths with standard deviations of less than 5 %, as shown in Fig. 5-6b.

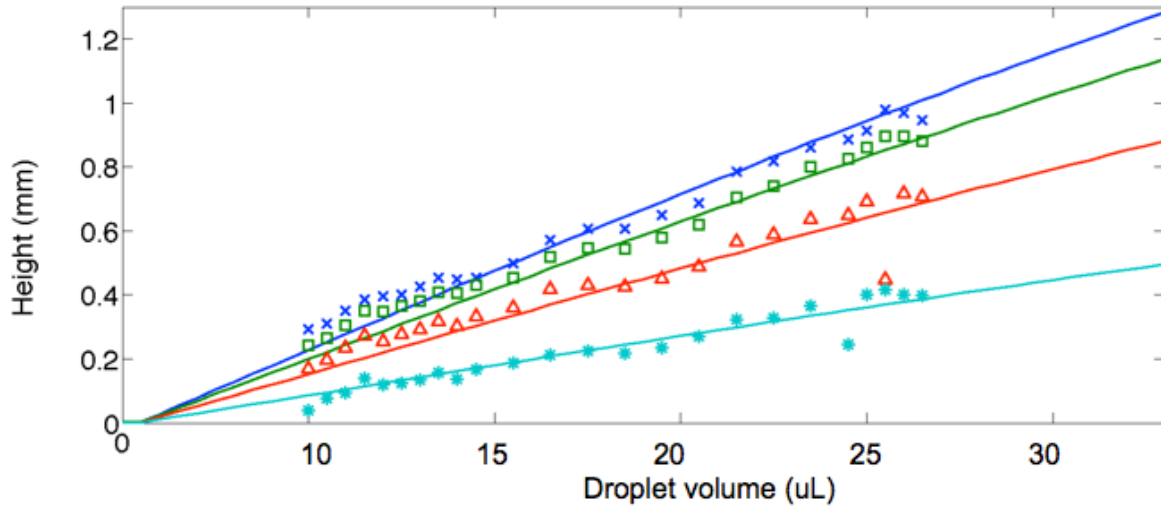
To further investigate the controllability of microneedle shape in the DBE method, the tip and bottom diameters of the samples fabricated in the previous section were measured and plotted in Fig. 5-6c. The preliminary results showed that the base diameter of the microneedle was mainly determined by the diameter of the mask aperture and did not change with different droplet volumes, while the tip size showed a strong correlation with the change of droplet volume.

5.5 Conclusion

The DBE method for making slanted solid and hollow microneedles was designed, developed, and characterized in this chapter. The results have shown that the three key parameters of the microneedle (the length, tip and base diameters) can be controlled precisely by manipulating the droplet volume and the mask design (the aperture diameter and the distance from the center). The measurements of these parameters were consistent with the analytical estimation, demonstrating



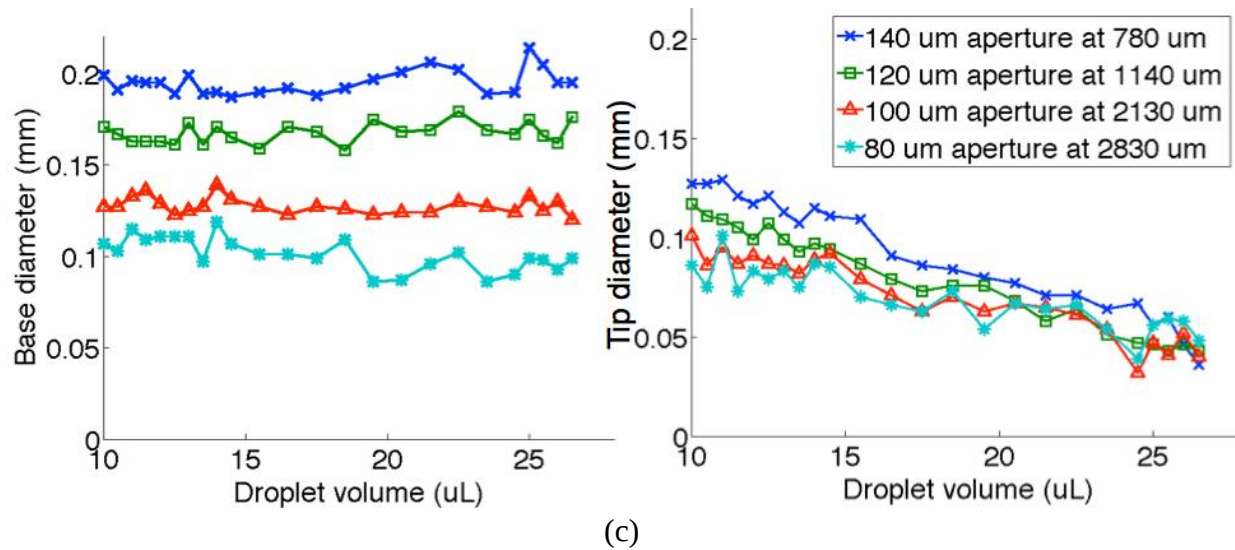
(a)



(b)

Figure 5-6. Dimension control of the DBE method: (a) comparison between the estimated heights and the measured heights of the fabricated microneedles with the standard deviations of less than 5%. (b) Estimated and measured heights of microneedles formed at different distances from the center of the droplet: 780 μm , 1440 μm , 2130 μm , and 2830 μm . (c) Dependency of base (left) and tip (right) size on SU-8 droplet volume. This experiment tested microneedles fabricated using four aperture diameters of 140, 120, 100, and 80 μm , at 780, 1140, 2130, and 2830 μm distance from the center of the droplet, respectively, with 24 different volumes ranging from 10 μL to 27 μL .

Figure 5-6 (cont'd)



the repeatability and controllability of the DBE method for fabricating varying-length microneedle.

In addition, flexibility and practicability of the DBE technique were proven with two adapted processes, by which a slanted hollow microneedle array and a slanted 3-D μ -waveguide array coupled with μ LEDs, presented in Chapter 4, were realized.

CHAPTER 6

Development of a cortically based hybrid visual prosthesis

6.1 Cortically based visual prosthesis

Based on works presented above, this author aims to develop a cortically based opto-epidural visual prosthesis for therapeutic management of all forms of blindness. Blindness has affected more than 40 million people worldwide [149] and approximately 1.2 million Americans [150]. In the United States, the annual cost of healthcare for individuals with visual impairment and blindness is over \$5.48 billion and the total economic impact is estimated at nearly \$51.4 billion annually [151]. Unfortunately, a solution to cure severe vision loss and blindness has not been found. Visual prostheses provide a potential clinical intervention to restore basic visual function of blind individuals through electrical stimulation of residual neurons in the visual pathway. Among many visual prostheses, the retinal prosthesis represents the most advanced technique and several such devices have entered clinical trials [152]-[160]. While significant improvement in visual function has been proven with the existing retinal prosthetic devices, these are only effective in treating blindness with functioning retina (e.g. age-related macular degeneration and retinitis pigmentosa). Alternative approaches are needed for the treatment of a large blind population where the retina and/or the optic nerve are severely damaged as in glaucoma (second leading cause of blindness (12.3 %) in the world), diabetic retinopathy (4.8 %), and trauma-induced blindness [161].

A cortical prosthesis bypasses the retina and the optic nerve, thus offers a general therapeutic opportunity for all causes of blindness. Early attempts at the cortical visual prosthesis were made by placing epidural electrodes on the visual cortex, electrical stimulation of which elicited phosphenes [20], [162]. Major shortcoming of the epidural stimulation is the high current needed to elicit phosphenes (in mA), resulting in poor spatial resolution, discomfort, and even focal epileptic activity [163], [164]. Intracortical electrodes can enable a reduced current threshold and improved spatial resolution of phosphene generation [165]-[167], but the invasive implantation of the penetrating probes increases the risk of stroke, hemorrhage, and infection. As mentioned in Chapter 2, inevitable limitations of electrical stimulation including non-specificity, unpredictable current pathway, electrical artifact, and electrode degradation, also increase the demand for a new technology [21].

This author hypothesizes that epidural optical stimulation of a large population of ChR2 expressing neurons in the primary visual cortex (V1) can induce phosphenes that lead to artificial prosthetic vision. This hypothesis is inspired by the electrically-based cortical visual prosthesis and formulated based on recent studies showing that optical stimulation of ChR2 expressing neurons in monkey V1 induced phosphenes and visuomotor behavior [168], [169]. Despite this behavioral evidence, little is known about spatial and temporal characteristics of the V1 neural activities in relation to the light-evoked phosphenes that are the key steps toward realization of a cortically-based opto-epidural visual prosthesis for treating blindness.

To address this knowledge gap, the hybrid neural interface was utilized for multichannel, bidirectional interaction with a large population of neurons in the V1 to explain the underlying correlation between light-evoked neural activities in the ChR2 transfected V1 and phosphene induction using advanced signal processing techniques. In the preliminary work discussed in

Chapter 3, this author successfully had shown that the Opto- μ ECoG array enabled simultaneous optical stimulation and recording of neural activities with significantly improved spatial resolution. Using the Opto- μ ECoG array, *in vivo* animal experiments based on rodent models have been designed to study the correlation between neural activities in the V1 and light-evoked phosphenes. Such experiments include 1) stimulus-driven ECoG data analysis; 2) a chronic device development for freely behaving animal experiment; 3) a behavioral test to analyze ECoG data in response to external visual stimuli; and 4) a behavioral test to examine the ability of optical stimulation of ChR2 expressing neurons to induce phosphenes. Several specific tasks must be done to prove the principal hypothesis, and details of the each task are discussed in the text below.

6.2 Major tasks for development of the hybrid visual prosthesis

6.2.1 Stimulus-driven ECoG data analysis

This author anticipated that multichannel ECoG data contain spatial and temporal information of neural ensemble encoding in response to a specific stimulus. These time-dependent spatial activation patterns are named ECoG snapshots. Based on this idea, an acute animal experiment was conducted to collect visual stimuli-driven ECoG data over the entire V1 through a high-density ECoG microelectrode array. Analysis of these data will furnish better understanding of underlying correlation between visual stimulus and the ECoG snapshots. Preliminary analysis results of stimuli-driven ECoG data are presented later in this chapter.

6.2.2 Chronic versions of the hybrid neural interfaces

Since the perception of artificial visual sensation in the animal model only can be verified in freely behaving animal experiment, development of chronic wireless neural interface is a critical step toward project success. Two different types of chronic devices have been developed based

on the flexible μ LED array. First, this author is working on the extension of the slanted waveguide array to make wireless hybrid neural interface using the inductive powering system based on switch-capacitive stimulation (SCS) system developed by the collaborators at Georgia Institute of Technology [170] . This author and the collaborators have designed the SCS system fully integrated with the LED array on a single flexible PCB board, and short-term and long-term reliability tests of the SCS-based wireless system is ongoing at time of this writing.

Another chronic device has been designed for a freely behaving experiment for mice. This battery-powered μ -LED array was designed for a small animal model that cannot carry an external device. Three versions of the device are available depending on requirements of experiments, and *in vivo* chronic animal experiment is in progress to evaluate long-term reliability test of the devices. Current developments of the chronic wireless hybrid neural interfaces are presented in Chapters 7 and 8.

6.2.3 Design of a behavioral animal experiment

As a proof of concept, a simple behavioral test was designed to verify perception of phosphenes using a rat model as shown in Fig. 6-1. First, in the visual cue association test, a single visual cue will be given to the rat. Within a certain time, the rat has to press a lever to receive a reward. If the rat does not press the lever after the cue, then no reward will be given. Once the animal is able to associate with the vision cue and the lever press task, then the Opto- μ ECoG array is implanted on the V1. After recovery from implant surgery, the subject will be back to the training protocol. This time the vision cue will be replaced with epidural light stimulation using the Opto- μ ECoG array, and the behavioral score will be recorded. If the rat presses the lever, it is possible that the epidural light stimulation induces phosphenes. Once the first test is successful, via more complicated pattern discrimination test will be tested.

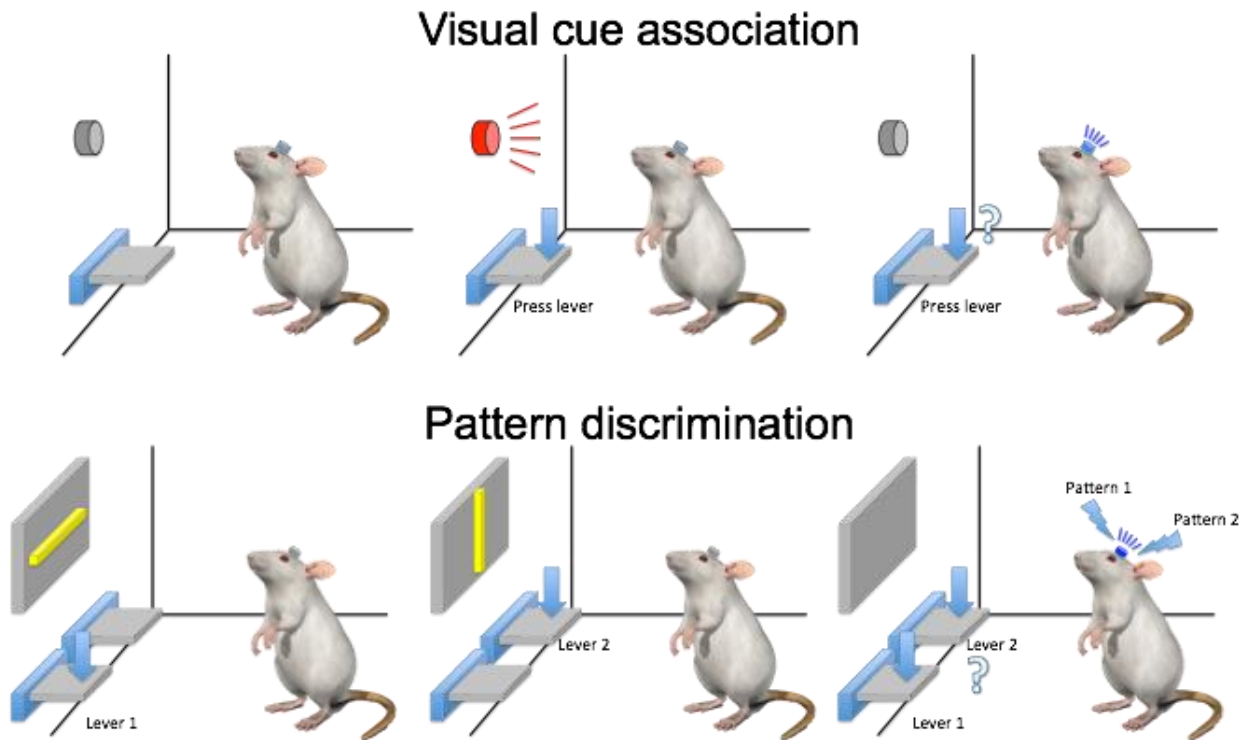


Figure 6-1 Proposed behavioral animal experiments to test artificial visual perception using cortically optical neural stimulation of the rat V1.

In this test, two distinguished vision cues are given, and each cue is associated with a lever. For example, cue 1 (horizontal line) is associated with lever 1, and the rat must press lever 1 to receive a reward when cue 1 is present. Similarly, cue 2 (vertical line) is connected with lever 2, and pressing only lever 2 will deliver a reward. During the training, neural activities (ECoG snapshots) during presence of cue 1 and cue 2 will be recorded, and data will be analyzed to find the stimulation parameter to reproduce the neural activity induced by the two different vision cues. After estimating the stimulation parameter during training session, vision cue will be replaced with epidural light stimulation using the Opto- μ ECoG array, and the behavioral score will be recorded. This author is aware of the high-risk nature of the new approach and proof-of-concept animal model studies, because it is not known whether simultaneous optical modulation of multiple neural populations can evoke visual sensation. So the chronic device will

be first tested in a well-established reflexive behavioral model, termed the gap-prepulse inhibition of the acoustic startle (GPIAS). More details about the behavioral experiments are discussed in Chapter 8.

6.3 Investigation of phase-locked neuronal oscillation

As a part of the major tasks for development of the hybrid visual prosthesis, stimuli-driven neural activity was first studied. The effects of optical stimulation in optogenetics have been studied in many aspects such as synaptic connections, behavioral responses, and corticocortical and corticothalamic connections [171]-[173]. However, in-depth understanding of characteristics of an optically-evoked large population of neuronal activity is not available. Neuronal oscillation, a fundamental component of brain function, is a synchronized synaptic activity across the local population of neurons. It can be interpreted into specific spatiotemporal patterns revealing how information flows through the brain [174]. It is believed that neuronal oscillation plays a critical role in large-scale functional integration enabling communication in large neuronal networks [175]. In general, a synchronized activity of large neuron populations across broad brain regions generates slower oscillations, while smaller localized neuronal assemblies are associated with faster oscillations [176]. ECoG recordings measure those brain activities directly with relatively higher spatial resolution, broader bandwidth, and higher amplitude, compared to noninvasive methods such as electroencephalography (EEG) and magnetoencephalography (MEG) [174].

Using the Opto- μ ECoG array, neural oscillations induced by natural and optical neural stimulations in an optogenetic rat model were studied. Phase synchronization of the recorded ECoG signals were analyzed using a new phase estimation method, based on the reduced interference distribution time-frequency phase synchrony (RID-TFPS) measure [177] and a

conventional instantaneous phase estimation based on the Hilbert Transform. The new method offers a phase estimation with uniformly high time-frequency resolution that can be used for defining time and frequency dependent phase synchrony. In the following sections, a short description of the RID-Rihaczek distribution-based phase synchrony measure is introduced. Next, *in vivo* experiments for stimuli-driven ECoG recordings are presented. Finally, data analysis results are presented and discussed.

6.4 Phase synchrony estimations

Existing measures to quantify neural connectivity from multichannel physiological data can be categorized broadly into linear and nonlinear measures [178]. Linear measures such as temporal correlation, spectral coherence, directed transfer function (DTF), partial directed coherence (PDC), and Granger causality, suffer from the assumption that underlying signals are stationary and the nature of the interactions between neuronal oscillations is linear. Nonlinear measures, on the other hand, attempt to address this limitation through phase synchrony [179], mutual information, and generalized synchronization measures. Recently, Aviyente et al., introduced a new time-frequency phase synchrony measure based on a complex energy distribution, referred to as Reduced Interference Rihaczek Distribution (RID-Rihaczek) as follows:

$$C(t, \omega) = \iint \exp\left(-\frac{(\theta\tau)^2}{\sigma}\right) \exp\left(j\frac{\theta\tau}{2}\right) A(\theta, \tau) e^{-j(\theta t + \tau\omega)} d\tau d\theta \quad (7)$$

where $\exp\left(-\frac{(\theta\tau)^2}{\sigma}\right)$ is the kernel function (in this case the Choi-Williams kernel), which is used to filter out the cross-terms due to multiple signal components in the original Rihaczek distribution, and $\exp\left(j\frac{\theta\tau}{2}\right)$ is the kernel function of the Rihaczek distribution [177]. $A(\theta, \tau) = \int x\left(u + \frac{\tau}{2}\right) x^*\left(u - \frac{\tau}{2}\right) e^{j\theta u} du$ is the ambiguity function of the signal. The spread of the

window σ is inversely proportional to f and determines the frequency of time-varying phase estimates.

Based on this new time-frequency distribution, the phase difference between two signals can be defined as

$$\Phi_{12}(t, \omega) = \arg \left[\frac{C_1(t, \omega) C_2^*(t, \omega)}{|C_1(t, \omega)| |C_2(t, \omega)|} \right] \quad (8)$$

Once the phase difference is quantified, phase synchrony can be computed. In this work, this author focuses on the intertribal phase synchrony that quantifies the phase locking to the response across trials. A phase locking value (PLV) is defined as

$$\text{PLV}(t, \omega) = \frac{1}{N} \left| \sum_{k=1}^N \exp \left(j \Phi_{11}^k(t, \omega) \right) \right| \quad (9)$$

where N is the number of trials and is the time-varying phase estimate between a reference trial and the k th trial/realization.

This novel measure has been shown to provide better time-frequency resolution in tracking the time-varying phase synchrony and increased robustness in noise compared to existing methods [177].

6.5 *In vivo* recordings

In vivo acute animal experiments were conducted to collect stimuli-driven ECoG recordings. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University. Prior to the acute experiments, rodent subjects (Sprague-Dawley rats: 250-400 g) had neurons in their V1 transfected with ChR2 to enable light sensitivity. For device implantation, the transfected rat (2 - 4 weeks after virus injection) was anesthetized with a mixture of ketamine/xylazine and placed in a stereotaxic apparatus. The Opto- μ ECoG array was surgically implanted over V1 and specific tests were conducted as described below.

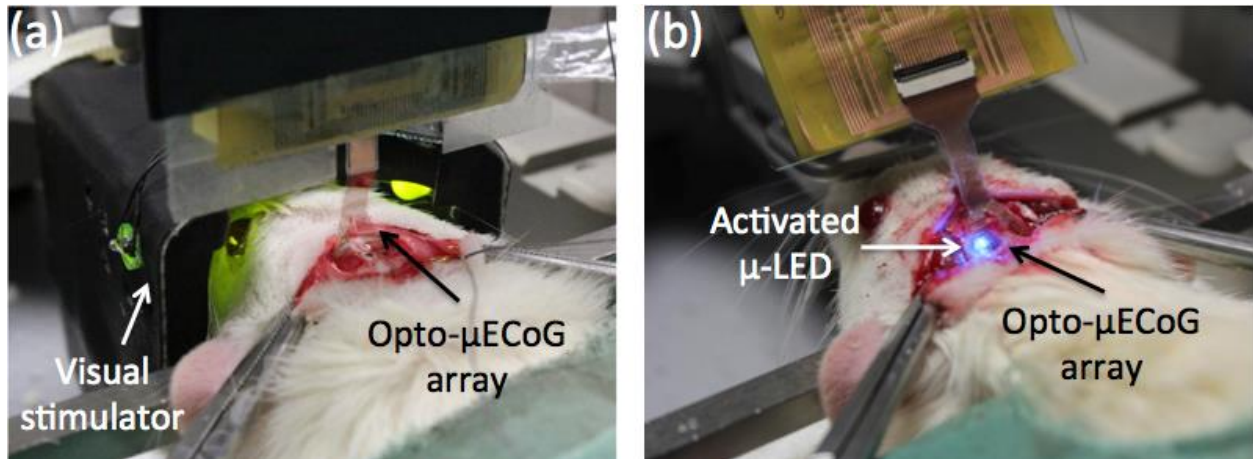


Figure 6-2. *In vivo* tests with (a) a natural visual stimulation and (b) a cortically optical stimulation.

Visual stimulus-driven ECoG signals were recorded first with a custom-made visual stimulator. Two green LEDs (a peak wavelength at 560 nm) were embedded inside a plastic mask to shield ambient light from rat eyes. The mask was installed on a stereotaxic (Fig. 6-2a) and a visual stimulus was delivered to the rat's left eye with 10 ms duration in a 1 s trial. ECoG signals were recorded from both primary visual cortices (V1) through the selected 23 channels (11 channels from right V1 and 12 from left V1) of the Opto-μECoG array.

The second experiment was performed to record cortically optical stimulus-driven ECoG recordings. In this case, the blue μ-LED (a peak wavelength at 460 nm) array was placed on the rat V1 (Fig. 6-2b) epidurally to optically modulate the neural activity with a light power density of $\sim 10 \text{ mW/mm}^2$ and a light pulse duration of 10 ms. ECoG signals induced by the μ-LED light stimulation were recorded simultaneously through 15 selected ITO electrodes. The recorded signals were amplified using an RHA2000 amplifier array then analyzed using MATLAB.

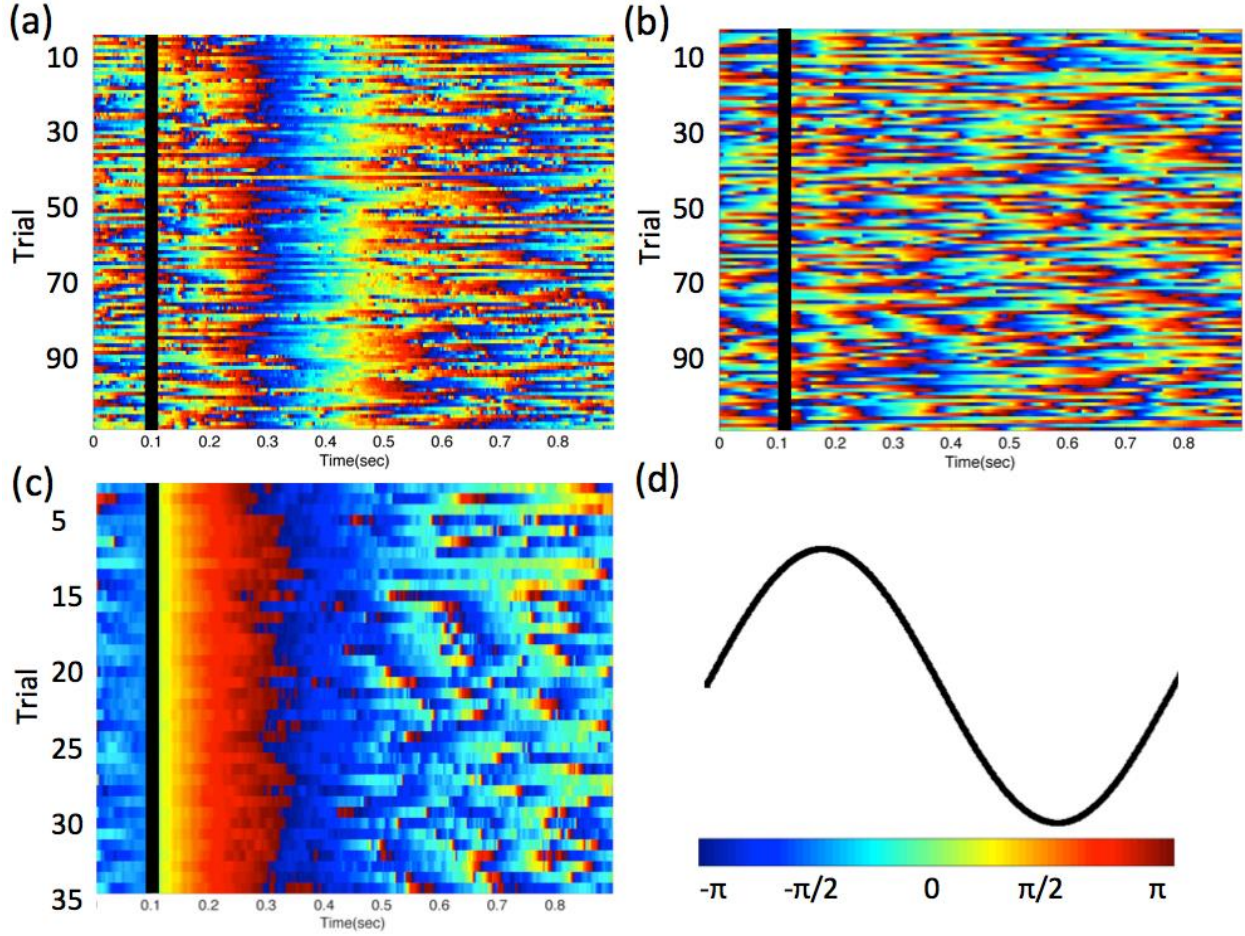


Figure 6-3. Instantaneous phase measure across trials based on Hilbert transform of the ECoG signals bandpass filtered at 4 – 8 Hz: (a) Visual I (106 trials at channel 4), (b) Visual II (114 trials at channel 4), and (c) Opto I (38 trials at channel 10). The black line on (a), (b), and (c) indicates stimulation ON. (d) Instantaneous phase and their corresponding color codings are shown.

6.6 Results

Three types of ECoG signals were recorded with the natural visual stimulation and the cortically optical stimulation via the Opto- μ ECoG array. The first dataset (Visual I) was recorded from the right V1 with the left eye stimulation (green LED) with 10 ms duration in a 1 sec trial (total of 106 trials). The second dataset (Visual II) was recorded from the left V1 with the same stimulation settings as Visual I (total of 114 trials). The third dataset (Opto I) was recorded from the right V1, evoked by the cortically optical stimulation via a single μ -LED, with an activation

voltage of 2.8 V (irradiance of 10 mW/mm²) for a pulse with a 10 ms duration in a 1 sec trial (total of 38 trials). All datasets originally were recorded at a sampling frequency of 25 kHz and down sampled to 1 kHz for data analysis. Each trial was truncated to a 100 ms pre-stimulation period and an 800 ms post-stimulation period, aligned based on the starting of the stimulus ON.

The Hilbert transform of each trial of ECoG recordings was computed to estimate the instantaneous phase. The recorded data of each trial was bandpass filtered to extract the signal around a frequency of interest (theta band: 4 – 8 Hz), then the Hilbert transform was applied to compute instantaneous phases of the signal. To visualize how instantaneous phases were modulated by different stimulations, the instantaneous phases of each trial, labeled with different colors according to Fig. 6-3d, were aligned based on the stimulus ON time and stacked. Both Visual I (Fig. 6-3a) and Opto I (Fig. 6-3c) datasets demonstrated a clear phase synchronization in theta band, because the phase values were modulated by the stimulus time. This modulation was extremely reliable across trials during the stimulation compared with the phases of each trial in Visual II (Fig. 6-3b). Two observations were made from the Hilbert transform analysis, which were validated through all channels with a nonparametric test ($p < 0.05$, Wilcoxon signed rank test for each pair of channels).

- The visual stimulation generated multiple time windows of phase synchronization during the trial, between 0.2 sec and 0.6 sec. The first phase synchronization event emerged around 100 ms after the stimulation, which is consistent with 100 ms latency observed in human vision system [180].
- The cortically optical stimulation generated almost deterministic phase-locked neuronal oscillations without any latency for ~ 0.4 sec. The duration of the phase-locked neuronal oscillation was ~ 400 ms, similar to that of the visual stimulation.

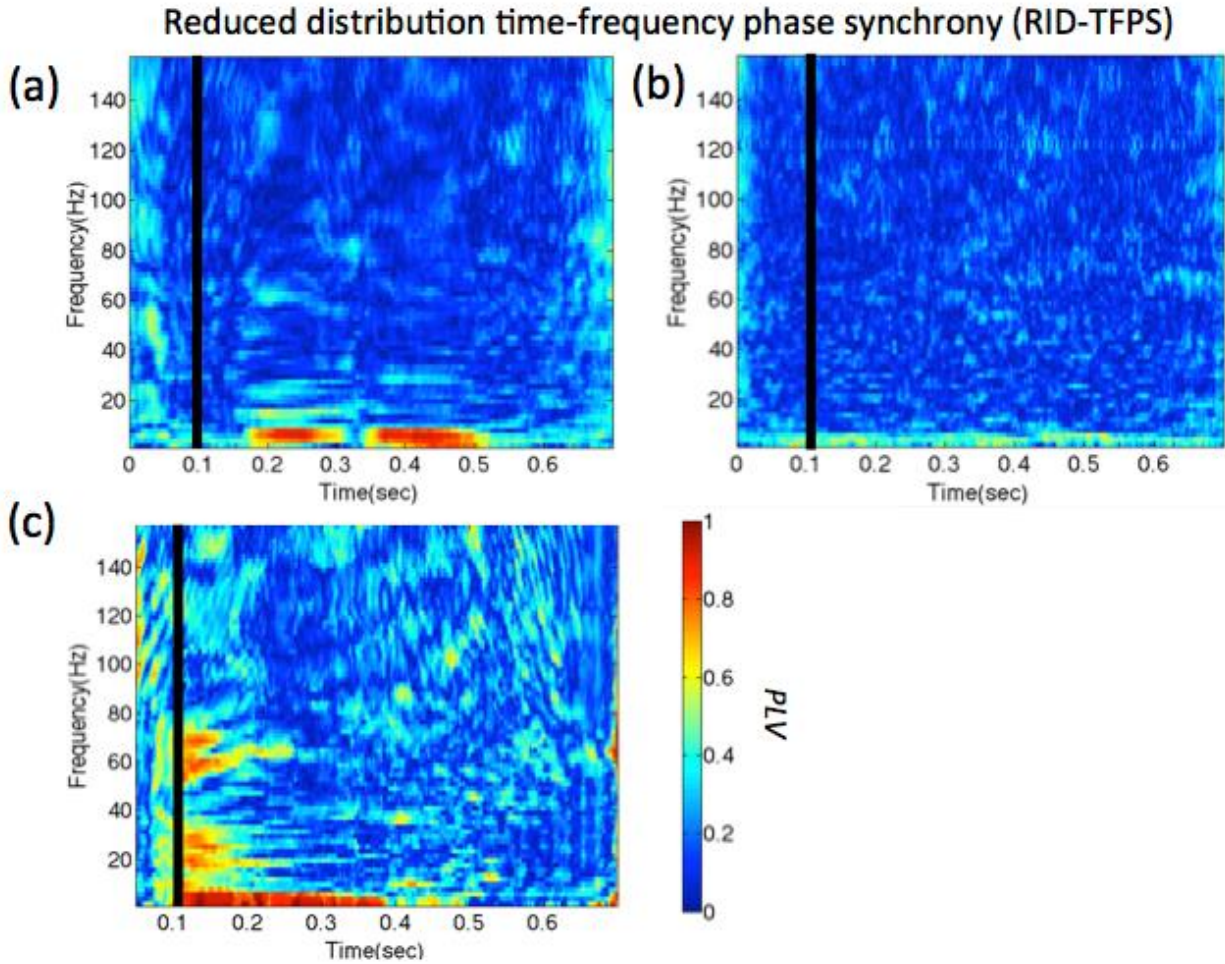


Figure 6-4. Broadband (1 – 150 Hz) phase-locked synchrony measured by RID-TFPS: a black line indicates stimulation ON. Phase-locked synchrony measures from (a) Visual I, (b) Visual II, and (c) Opto I.

Second, phase synchrony of three datasets in a broadband frequency range was measured using the RID-TFPS method. The analysis results of selected channels, channel 4 from Visual I and channel 8 from Opto I, are shown in Fig. 6-4a and c, respectively. High phase-locked synchrony values in a low frequency band (1 – 25 Hz) in both Visual I and Opto I datasets were consistent with the phase-locked oscillations observed in the previous analysis based on the Hilbert transform. Because the RID-TFPS approach can monitor a phase-locked synchrony throughout the broadband frequency range without bandpass filtering, synchronization in different frequency bands was detected. For example, for the dataset Visual I, there is increased

synchrony (Fig. 6-4a) ~ 0.08 sec after the simulation, spanning multiple bands such as theta band (4 – 8 Hz) ($p < 0.01$, Wilcoxon signed rank test for each pair of channels), alpha band (9 – 12 Hz) ($p < 2.3e-4$, Wilcoxon signed rank test for each pair of channels), and beta band (13 – 30 Hz) ($p < 0.03$, Wilcoxon signed rank test for each pair of channels), followed by later synchronization observed within lower frequency bands such as delta band (0.1 – 3.9 Hz) ($p < 2.1e-4$, Wilcoxon signed rank test for each pair of channels) and theta band ($p < 0.017$, Wilcoxon signed rank test for each pair of channels). Several observations were also made from the analysis results.

- The phase-locked synchrony estimation using RID-TFPS is consistent with the findings in the previous method based on the Hilbert transform.
- Phase-locked neuronal oscillations in multiple bands (theta, alpha, and beta bands from Visual I and theta, beta and gamma bands from Opto I) were observed.
- 100 ms latency was observed from Visual I, while immediate phase-locked oscillations were observed from Opto I.
- Stronger and more frequent phase-locked synchronies in gamma band were observed from Opto I than counterparts from Visual I.

6.7 Conclusion

The influence of cortically optical stimulation on a large population of neurons in rat V1 was investigated by studying differences between visual stimulation and cortically optical stimulation evoked neuronal oscillations and their phase-locked synchrony measures. Simultaneous cortically optical stimulation and light-evoked ECoG recordings were done using a newly developed Opto- μ ECoG array. A new time-varying phase synchrony measure, referred to as reduced interference distribution time-frequency phase synchrony, allows detecting a phase-

locked synchrony throughout a large range of frequency bands without bandpass filtering. Preliminary results revealed reliable and consistent phase-locked synchrony on both the visual and cortically optical stimulations. Differences in phase modulations between the natural stimulus driven neural oscillations and the optically stimulated neural oscillations were also studied, in terms of latency and specific frequency bands of phase-locked synchrony.

CHAPTER 7

Chronically implantable wireless hybrid neural interfaces

7.1 A wireless slanted optrode array

Building on the previous studies, this chapter presents a wireless slanted optrode array with integrated μ -LEDs, capable of simultaneous light stimulation and electrical neural recording to achieve a truly untethered bi-directional neural interface. Recording electrodes wrapped around the waveguide cores permit simultaneous measurement of neural responses from various depths upon optical excitation. Fig. 7-1 depicts the concept and design of the wirelessly powered-slanted optrode array, containing 32 embedded μ -LED light sources on a $2.5 \times 2.5 \text{ mm}^2$ flexible substrate, with 4×4 channels per each hemisphere to cover the bilateral visual cortices in rats. The slanted microneedle array was fabricated separately on a PDMS substrate and integrated with the multi-LED array using shape-matching assembly. A DBE method, discussed in Chapter 5 [130] was used to form a slanted microneedle structure, in which the lengths (300 – 1500 μm) of individual microneedles were determined by the height variance along the droplet curvature. A multilayer metal/Parylene shell was fabricated on the waveguide core to prevent photoelectrical artifact and improve biocompatibility.

Integrated LED light sources powered by the wireless switched-capacitor stimulator (SCS) enable a truly untethered system [33]. Inductive power transmission across the skin has been a viable solution to provide sufficient power to the implantable medical devices (IMDs), while overcoming size, cost, and longevity constraints of embedded primary batteries [33]. Light

sources, however, typically require high instantaneous power to emit sufficient light for optical neural stimulation, which can be a significant limiting factor in conventional IMDs [181]. Fig. 7-2a shows the conceptual block diagram of a conventional inductively-powered array of LEDs for wireless optogenetics, where a rectifier and a regulator convert AC voltage across a secondary coil, L_2 , to DC output voltage to supply a LED driver. Power losses in these stages result in poor overall power efficiency from L_2 to the LED. Moreover, high instantaneous power that flows to the LEDs when they are on, leads to large load variation, which affects the impedance matching with the inductive link, significantly increasing the required inductive power level, affecting a safety issue, and degrading the inductive link power efficiency as well as supply voltage for the rest of the IMD.

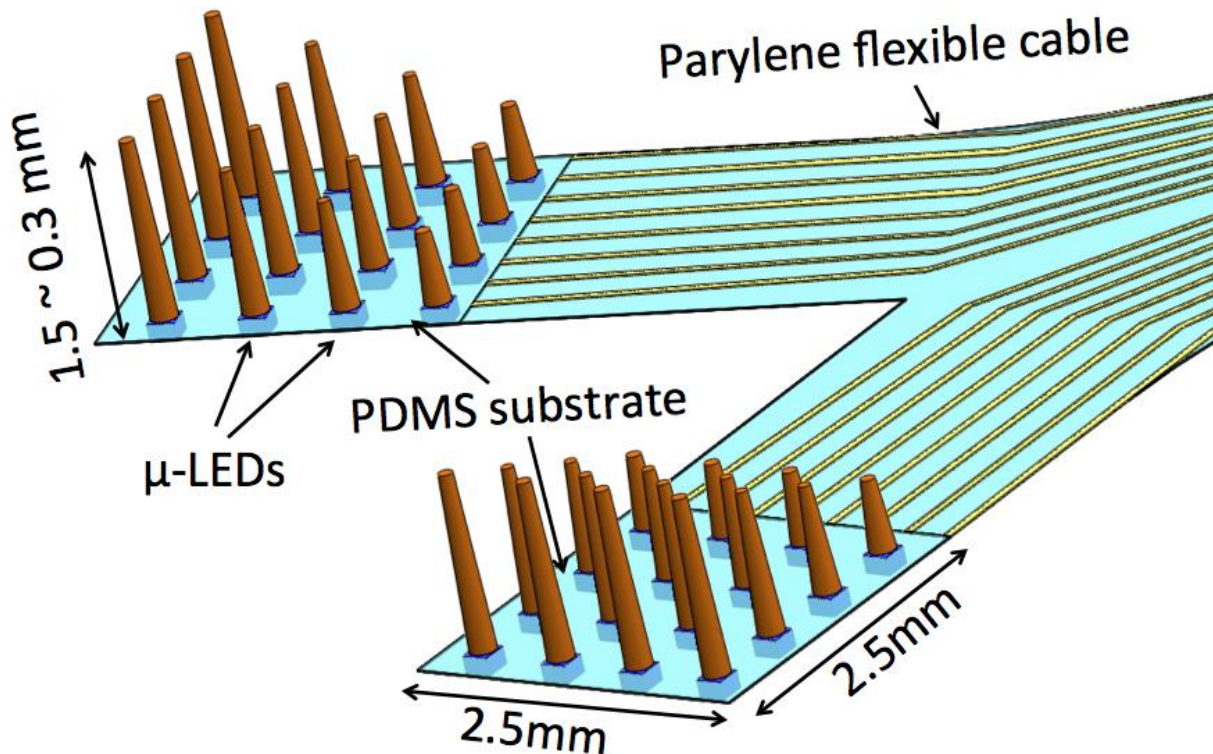


Figure 7-1. Concept diagram of wireless optrode array with integrated μ -LEDs

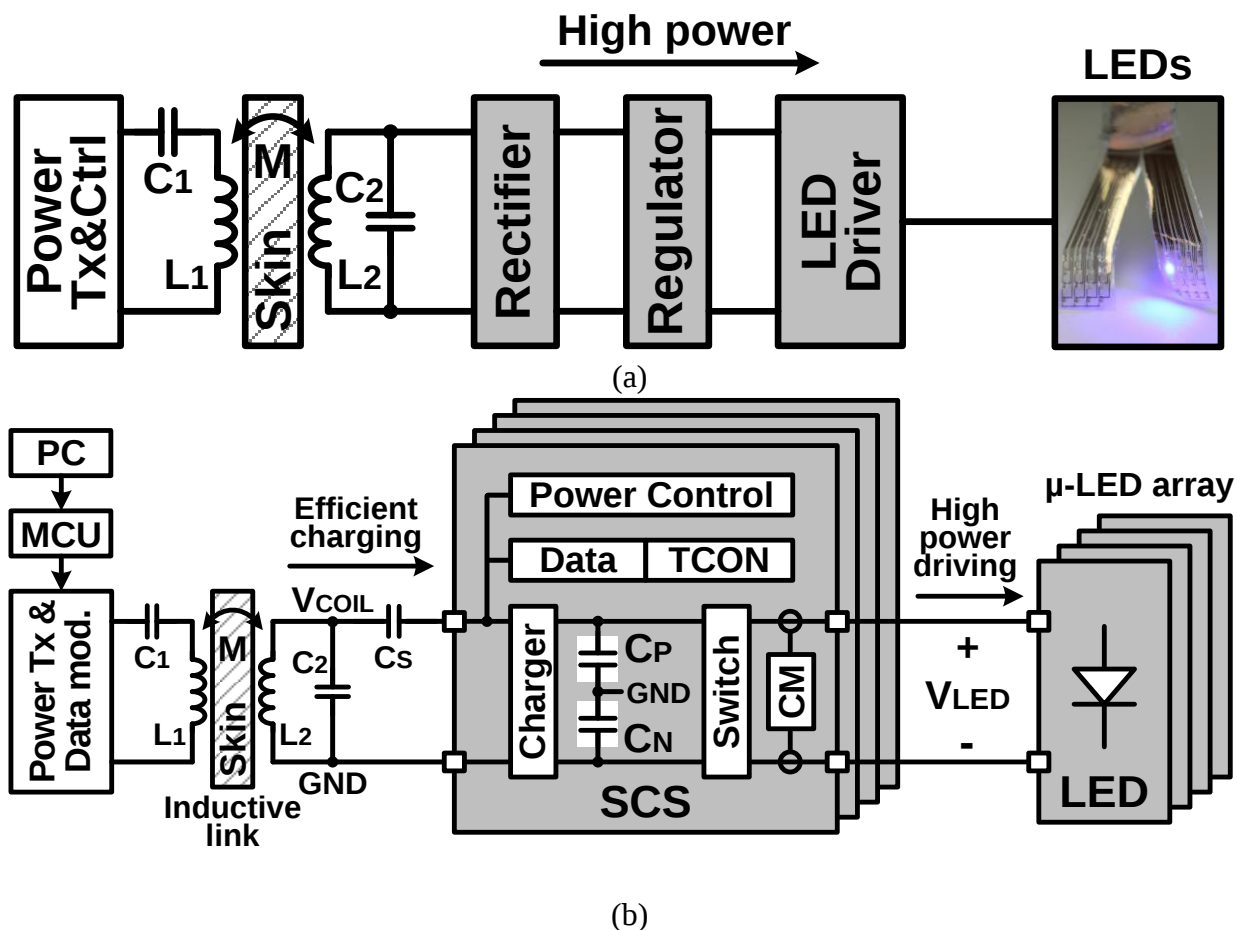


Figure 7-2. (a) Simplified block diagram of a conventional inductively-powered device combined with an array of LEDs for wireless optogenetics and (b) simplified block diagram of the wireless switched-capacitor stimulating (SCS) system to efficiently drive the μ -LED array.

To address these limitations in implantable optogenetics, Lee et al. has utilized a switched-capacitor stimulating (SCS) system for power-efficient wireless optical stimulation [33]. The SCS system efficiently charges a small array of storage capacitors directly from the inductive link and periodically discharges them into the micro-LED array, providing high instantaneous current without burdening the inductive link and system supply. To control stimulation parameters, a custom-designed PC interface wirelessly sends data to the SCS system through the inductive link, while a commercial neural recording system simultaneously detects the evoked neural signals from the same 3-D optrode array. Moreover, the proposed hermetic sealing method and *in vitro* test results verified the functionality and reliability of the chronic

implant prototype, which will be used to prepare the SCS system for implantable optogenetics. *In vivo* acute experiments were conducted to demonstrate optical modulation of neural activity with the integrated LED array powered by the wireless SCS system.

7.2 Methods

7.2.1 Slanted waveguide array

In Chapter 5, a polymer-based fabrication method (DBE) was introduced, utilizing the height variance in a dome-shaped SU-8 structure to create out-of-plane microneedles with various lengths, and the slanted optrode array also was designed based on the DBE method.

The optrode enables delivering light into the target area as well as recording neural activity simultaneously. The basic structure of the optrode comprises a SU-8 microneedle waveguide core covered by metallic cladding, which is used as a recording electrode and a shielding layer to prevent light-leakage from the sidewall. However, it is found that if the metal layer is used for neural recording directly, light-induced artifacts will occur, which require post processing to recover the original neural data. This phenomenon is consistent with a classical photoelectrochemical finding, the Becquerel photovoltaic effect. To minimize the light-induced artifacts, four layers of oxide-polymer-metal-polymer sandwich structure were designed. Multilayer metal/Parylene shell was fabricated on the outer sidewall of the waveguide core, which contained an indium tin oxide (ITO) shielding layer to prevent photoelectrical artifact, an opaque metal layer to block light side-leakage and record neural activity, and Parylene as insulating layers between ITO and metal and an encapsulation for improved biocompatibility. Opaque metal was removed from the tip of the waveguide to allow light delivery to the brain neurons. A profile of the sandwich structure is shown in Fig. 7-4b.

7.2.2 Switched-capacitor stimulation (SCS)

Fig. 7-2b shows the simplified block diagram of the wireless switched-capacitor stimulating (SCS) system for power-efficient optogenetics. Conventional inductively-powered current stimulators require a rectifier, a regulator, and an array of current sources to generate stimulation pulses from V_{COIL} , while power losses at each stage result in poor overall stimulator efficiency [33]. Contrarily, the proposed SCS system efficiently charges a bank of storage capacitors, CS , directly from V_{COIL} through the inductive link without using any rectifiers and regulators, improving the capacitor charging efficiency [33]. Moreover, the charge stored in capacitors is delivered to the load through switches, efficiently creating stimulation pulses.

In the inductively-powered SCS system, a power transmitter (Tx) drives the primary coil, L_1 , at the power carrier frequency, f_C . This signal induces current in the secondary coil, L_2 , through the inductive link, generating an AC voltage, V_{COIL} , across the resonance circuits, L_2 and C_2 . The SCS system efficiently charges a bank of storage capacitors, C_P and C_N , directly from V_{COIL} through a series capacitor, C_S , and an inductive charger without using any rectifiers and regulators [170], improving capacitor charging efficiency. The charge stored in capacitors is delivered to the load through switches, efficiently creating stimulation pulses. In addition, a charge monitoring (CM) circuit measures the amount of injected charge to enable charge-balanced stimulation. For power-efficient wireless optogenetics, the SCS system periodically discharges the storage capacitors into an array of micro-LEDs to emit sufficient light and evoke neural activity. After charging, C_P and C_N pairs are connected in series to provide higher LED voltage, V_{LED} , for optical stimulation.

Forward data telemetry utilizes the same inductive link to wirelessly set stimulation parameters in the system, and the on-chip timing controller (TCON) generates timing signals for

capacitor charging and LED driving. Hence, the SCS system can be implanted chronically for experiments with freely behaving animal subjects. The power control unit generates supply voltages for the rest of the system and prevents the systems from overvoltage due to sudden strong coupling.

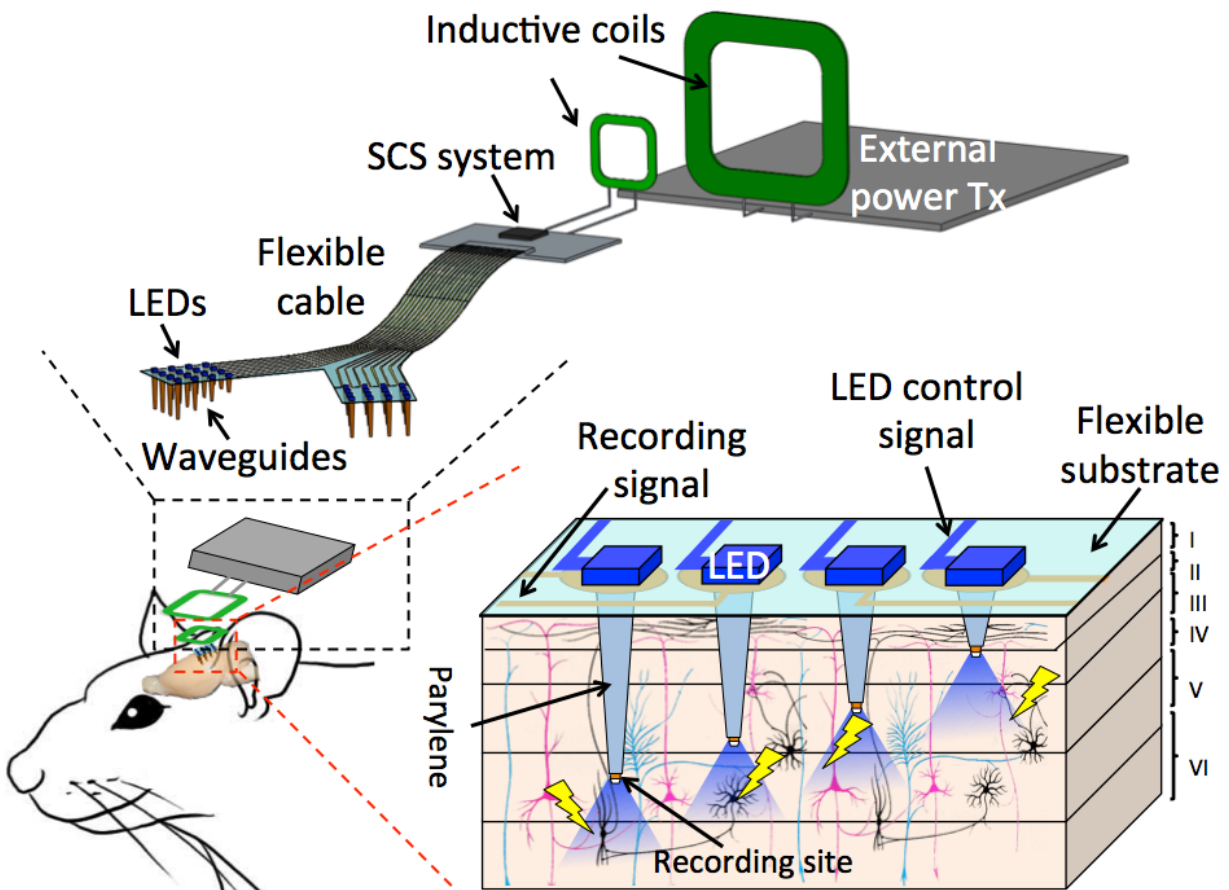


Figure 7-3. 3-D model for *in vivo* optogenetics experiments with the SCS system. Inset: Optrode array with micro-LEDs for optical stimulation and penetrating electrodes wrapped around the waveguides for neural recording.

Fig. 7-3 shows the 3-D model for *in vivo* wireless optogenetics with the SCS system, which receives wireless power and data through the inductive link. The SCS ASIC drives the 3-D flexible optrode array, which consists of micro-LEDs for optical stimulation, penetrating

electrodes for neural recording, and micro-needle waveguides to enable precise and efficient light delivery to the target tissue with high spatial resolution [32].

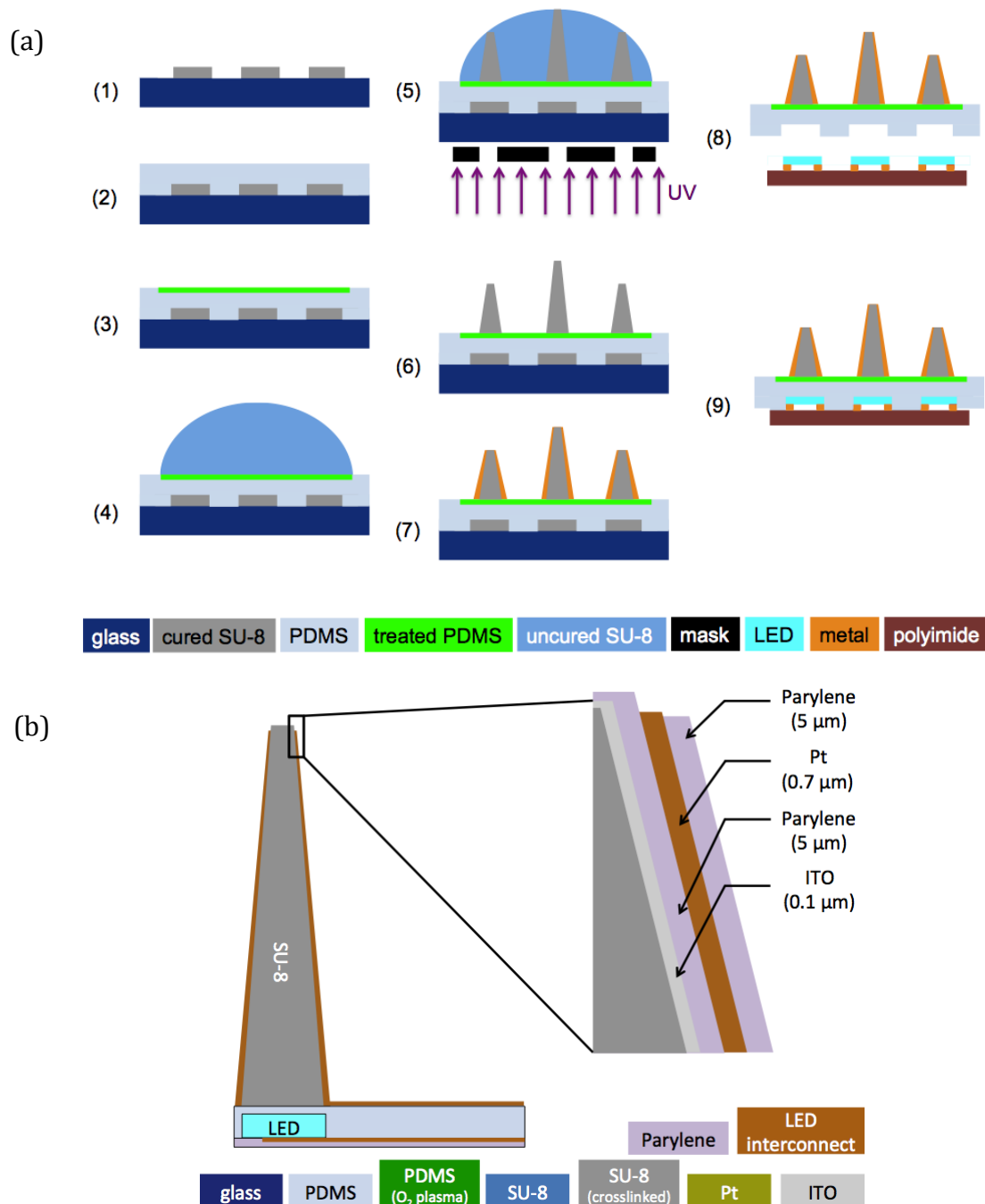


Figure 7-4. (a) Fabrication process flow for making the slanted optrode array with integrated μ -LEDs and (b) the profile of the optrode.

Neural signals are recorded from penetrating electrodes wrapped around the waveguide core and only exposed at the tips of the waveguides, through an independent recording setup. In this setup, the wireless SCS system with slanted optrode arrays enables simultaneous optical stimulation and electrical neural recording to test an untethered bi-directional neural interface.

7.3 Fabrication

To reduce fabrication complexity, the multi-LED array and the slanted optrode array were fabricated and calibrated separately. Then the system was assembled by polymer bonding of individual components with SU-8. Detailed fabrication was divided into two steps: flexible multi-LED array and slanted optrode array fabrication. Details of flexible multi-LED array fabrication process was described in Chapter 3.3.1, so, this study only focuses on the fabrication process of the slanted optrode array (Fig. 7-4a).

(1) A 3" glass wafer was cleaned and put through a dehydration bake, and $\sim 50\ \mu\text{m}$ SU-8 layer was spun onto the wafer and patterned as the mock LEDs. (2) A thin layer of PDMS was spun on the SU-8 master to create cavities matching the shape of the LED. (3) After the PDMS was cured for 40 min at $95\ ^\circ\text{C}$, photoresist (PR) was patterned on the PDMS substrate to expose 7 mm-diameter circles, followed by oxygen plasma treatment to convert the exposed hydrophobic areas to hydrophilic ones. (4) After removal of the PR mask, $\sim 45\ \mu\text{L}$ SU-8 (SU-8 3005) was dispensed on top of the plasma treated PDMS surface using a micropipette and (5) patterned with the backside exposure to form the microneedles. (6) After SU-8 development, the array was polished by O_2 plasma etching. (7) DC sputtering of a $0.1\ \mu\text{m}$ thick ITO layer was performed in a Kurt Lesker Axiss PVD System, followed by deposition of $5\ \mu\text{m}$ Parylene-C in a chemical vapor deposition (CVD) system (PDS2010 Parylene Coater, Specialty Coating System) and then $1\ \mu\text{m}$ thick Au layer in a thermal evaporator (Edward Auto306). Opaque metal was

removed using wet etching from the tip of the waveguide to allow light delivery to the brain neurons and 5 μm Parylene-C was deposited as a protection layer. (8) The Parylene-C film at the tip of the optrode was removed using reactive-ion etching (RIE), and the membrane was released from the glass wafer. (9) Finally, the microneedle array with the matched LED cavities was aligned onto the corresponding LED chips and bonded with polymer adhesive.

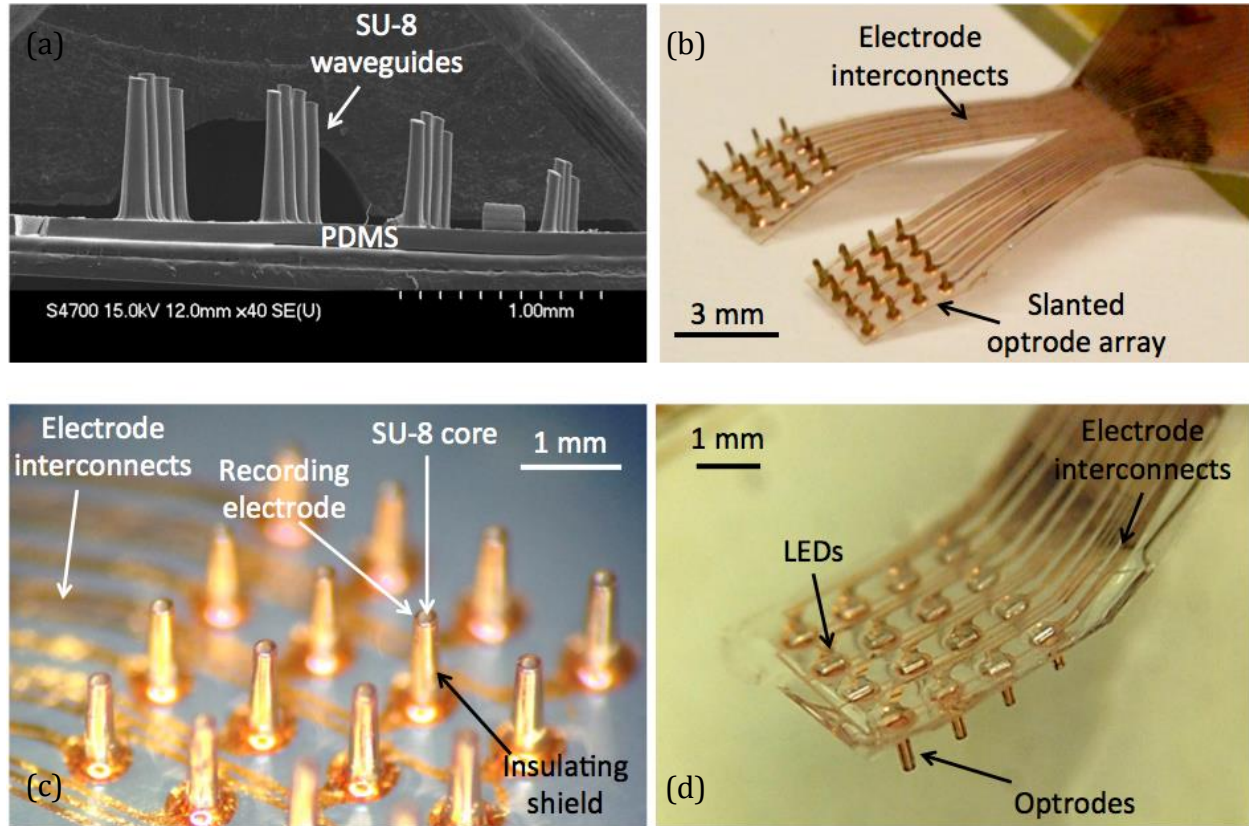


Figure 7-5. Fabricated prototypes of (a) SEM images of a slanted microneedle structure, (b) 32-channel optrode array, (c) microscopic image of individual optrode, and (d) optrode array coupled with μ -LEDs.

Fig. 7-5 shows images of the prototypes of slanted optrode array. SEM image of length-varying SU-8 microneedle structure (300 – 800 μm) shown in Fig. 7-5a demonstrated that the capability of the DBE method for making varying-length microneedles. The electrode-electrolyte interface impedances of the optrode array were measured at 1 kHz using a built-in electrode-impedance-testing circuitry in an Intan evaluation board (RHD2132 and RHD2000-EVAL, Intan

Tech. LLC, CA, USA). The impedance of the optrode array was ranging from 10 to 500 k Ω , which is suitable for local field potential recordings. In this study, the impedance of the optrode was controlled by size of the Parylene opening.

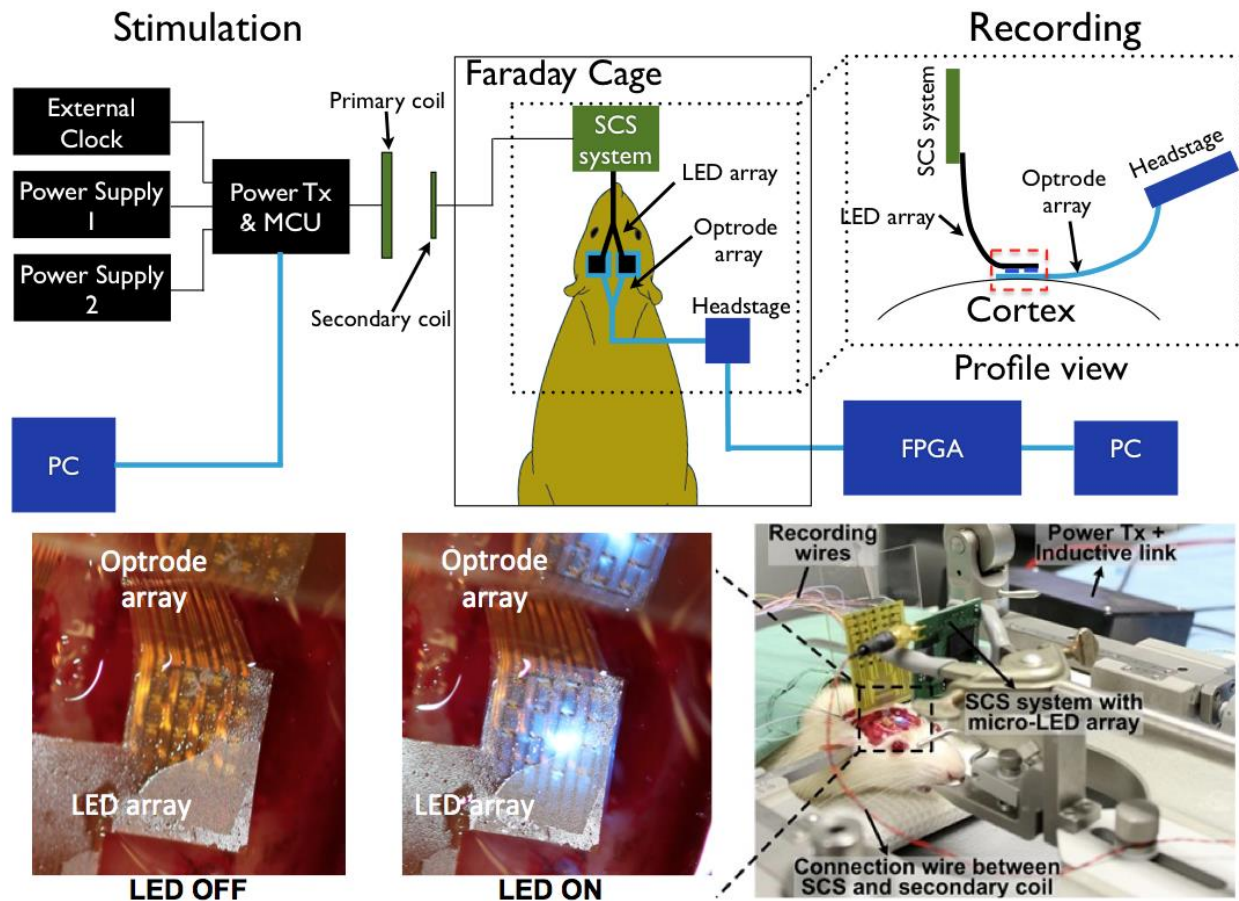


Figure 7-6. Schematic of *in vivo* animal experiment set-up with the wireless SCS system.

7.4 Results

7.4.1 *In vivo* animal experiments

In vivo acute animal experiments were performed to demonstrate the ability of the slanted optrode array to simultaneously modulate and record neural activities in the visual cortex (V1) of a rat. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University. Prior to the acute experiments, rodent subjects (Sprague-

Dawley rats: 250 – 400 g) had neurons in their V1 transfected with ChR2 to enable light sensitivity.

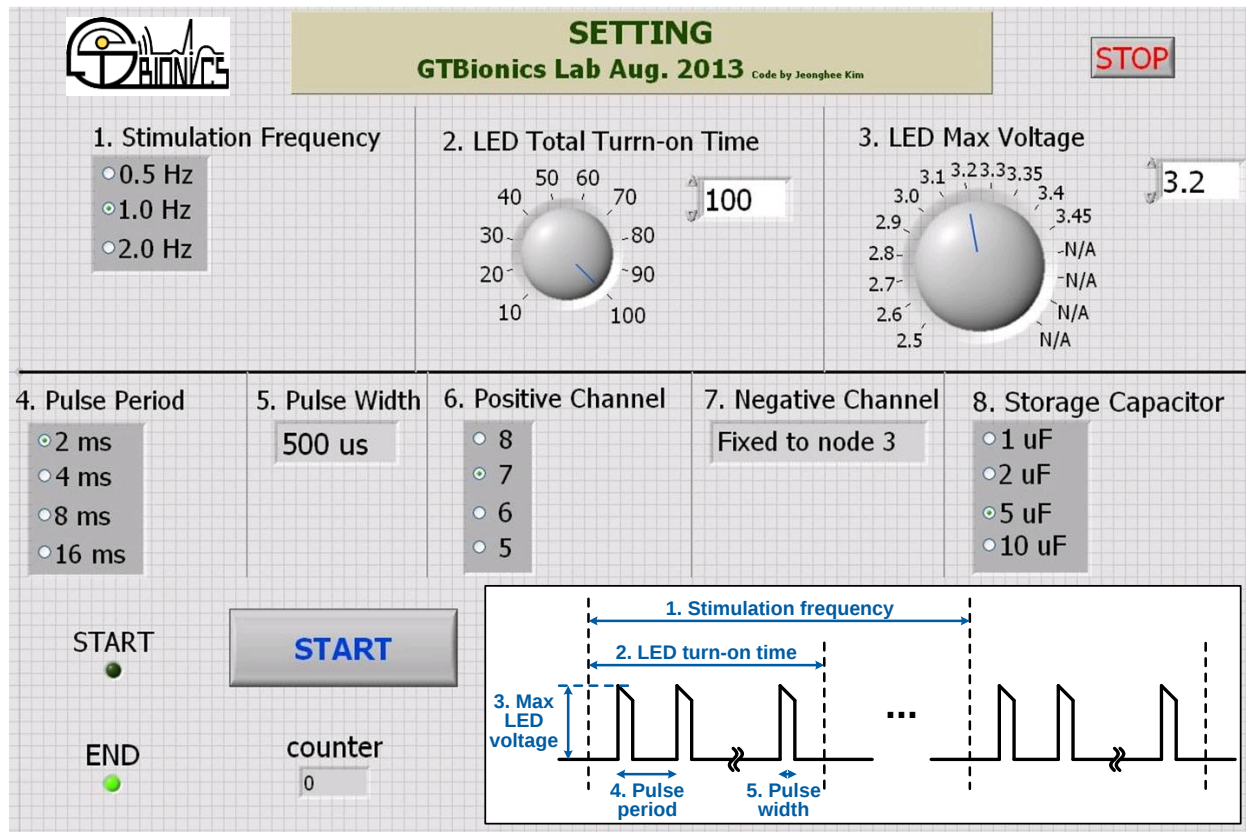


Figure 7-7. SCS system GUI in LabVIEW to wirelessly control the SCS parameters. Inset: LED driving voltage waveform and its controllable parameters.

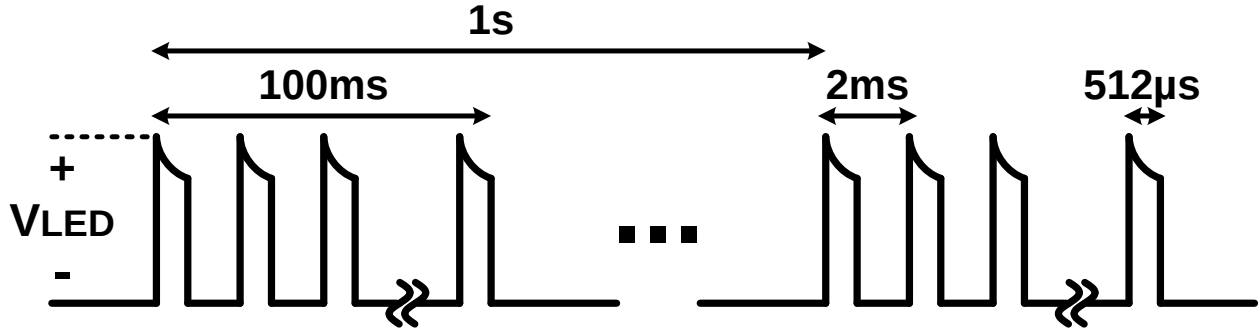
As shown in Fig. 7-6, the SCS system was used to power and control the 32-channel LED array and a 32-channel headstage was used for simultaneous extracellular recording. The SCS system receives wireless power and data through the inductive link and drives micro-LEDs in the optrode array. The SCS prototype occupies $3.9 \times 3.9 \text{ cm}^2$ on PCB, and includes off-chip components for testing and optrode connectors. Key parts of this prototype are the SCS chip ($5 \times 2.4 \text{ mm}^2$) and 4 pairs of $1 \sim 5 \text{ }\mu\text{F}$ off-chip storage capacitors (SMD-0402, $1 \times 0.5 \text{ mm}^2$ each). The SCS system can be shrunk further using a single flex-PCB that can connect directly to the LED array and include the receiver coil, L_2 , without connectors or testing components. Storage

capacitors also can be placed on the opposite side of the SCS chip, minimizing the PCB size for implantable optogenetics. Neural signals were recorded simultaneously from the penetrating electrodes on the same optrode array through an evaluation board and commercial hardwired recording setup (RHD2132, Intan Technologies, CA, USA).

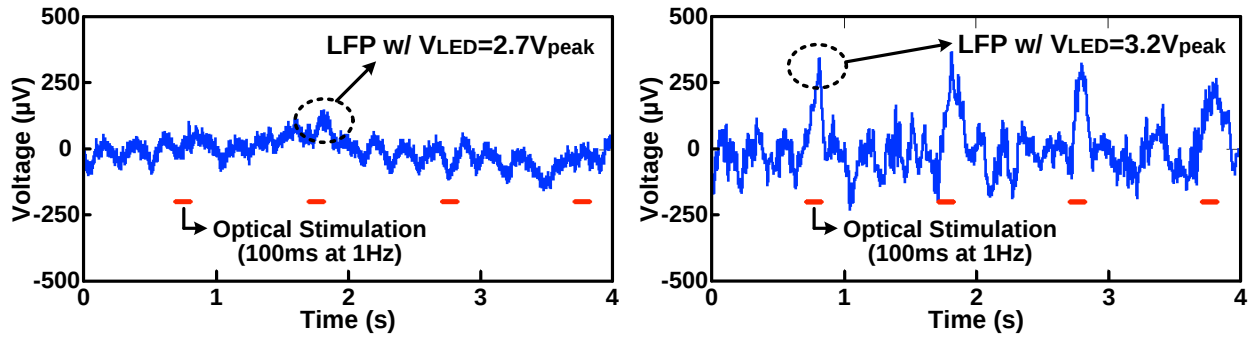
A graphical user interface (GUI) has been implemented in the LabVIEW environment to send data packets from the PC to the MCU of the power Tx module, as shown in Fig. 7-7. Several parameters of the LED driving signal can be adjusted in this setup: 1) stimulation frequency, 0.5 ~ 2 Hz, 2) LED total turn-on time, 10 ~ 100 ms, 3) LED peak voltage, 2.5 ~ 3.45 V, 4) pulse period, 2 ~ 16 ms, 5) pulse width, 512 μ s, 6) positive output connections, 4 channels, 7) negative output connections, fixed, and 8) storage capacitance, 1 ~ 10 μ F. While the pulse train periodically turns the LEDs on and off, the effective turn-on time can be calculated simply as total turn-on time x (pulse width / pulse period).

Fig. 7-8 shows the LED driving voltage, V_{LED} , for optical stimulation with SCS and light-induced *in vivo* LFP results. The LFPs below 500 Hz were recorded using an optrode array with waveguides in the rat's brain when the SCS system drove micro-LEDs with a 512 μ s pulse train for 100 ms at 1 Hz and $V_{LED} = 2.7$ V_{peak} and 3.2 V_{peak}, as shown in Fig. 7-8a.

A clear light-induced neural activity was observed in LFP (1 – 500 Hz) in time domain driven by 3.2 V_{peak} input voltage (Fig. 7-8b). 3.2 V_{peak} input voltage resulting in average irradiance of 1.4 mW/mm² at the tip of the optrode, which is above the minimal irradiance (1 mW/mm²) required for light-evoked neural response. This author also tried lowering the input voltage to 2.7 V_{peak} (resulting in the average irradiance of 0.35 mW/mm²), but no significant neural modulation was observed.



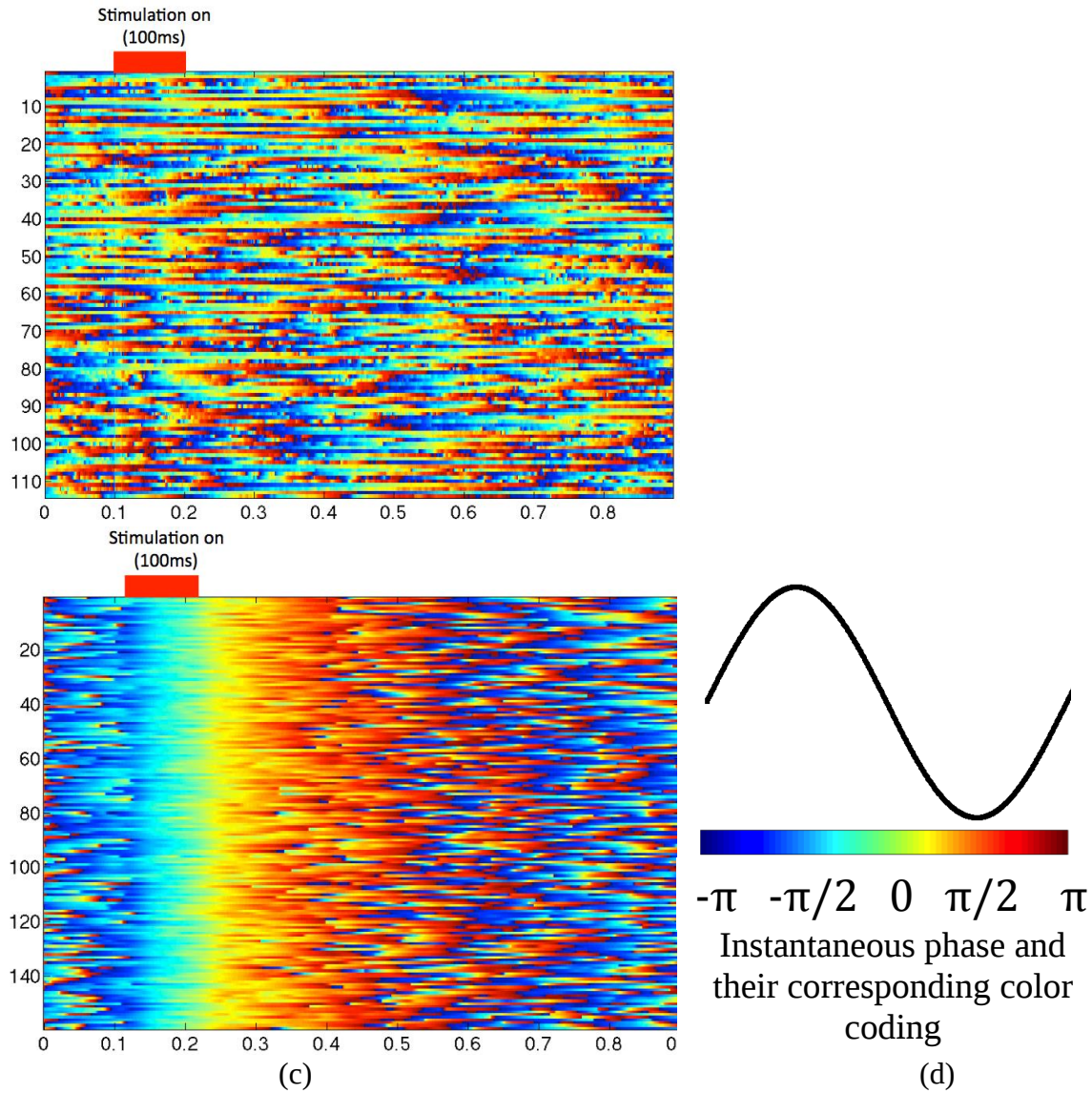
(a)



(b)

Figure 7-8. Light-induced LEP recording driven by 2.7 V_{peak} and 3.2 V_{peak} for 100 ms: (a) LED driving voltage, V_{LED} , for *in vivo* optogenetics with SCS (b) light-induced local field potentials (LFP) with $V_{LED} = 2.7 V_{peak}$ and 3.2 V_{peak} , (c) instantaneous phase with $V_{LED} = 2.7 V_{peak}$ (top) and 3.2 V_{peak} (bottom) in low frequency band (1-25Hz), and (d) their corresponding color-coding.

Figure 7-8 (cont'd)



Clearly to visualize neural oscillations induced by the optrode array, instantaneous phase of the two datasets (2.7 V_{peak} and 3.2 V_{peak} input voltages) of light-evoked LFP (1 - 25 Hz) was measured based on Hilbert transform. The instantaneous phases of each trial (1 s with 100 ms LED on), labeled with different colors according to Fig. 7-8d, were aligned based on the stimulus ON time and stacked. With 3.2 V_{peak} input voltage, light-evoked neural activity shows clear phase synchronization, while no phase synchrony was observed in neural recording data

with 2.7 V_{peak} input voltage. This experiment verified the feasibility of wireless optical stimulation and simultaneous recording via the wireless slanted optrode array.

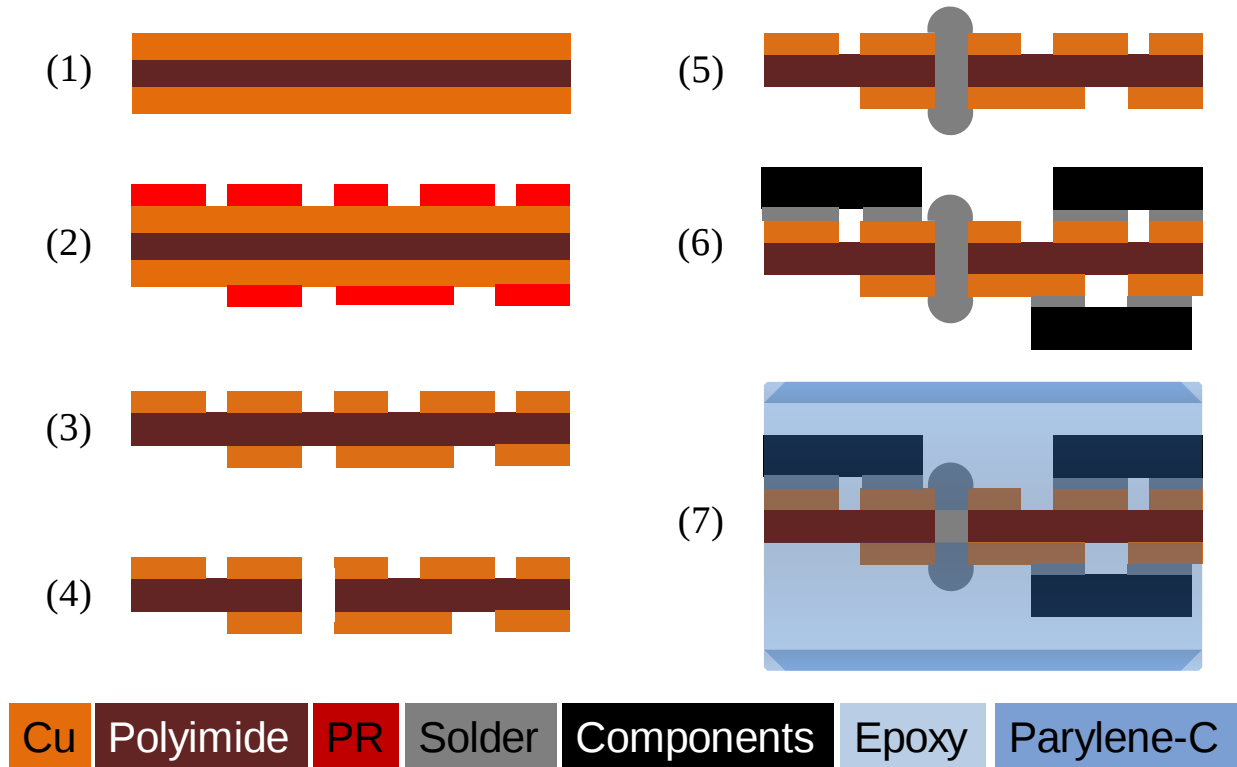


Figure 7-9. Fabrication steps of a hermetically-sealed chronic implant on a flexible polyimide circuit.

7.4.2 Hermetic sealing for chronic implants

Fig. 7-9 shows the fabrication steps of a hermetically-sealed chronic implant on a flexible polyimide circuit for experiments using freely behaving animals. The flexible polyimide circuit was fabricated using Pyralux®AP (AP7163E, DuPont) with the following steps: 1) A 3-inch Pyralux® wafer was cut and cleaned, and a 3-μm thick photoresist (PR) layer was spin coated. 2) The circuit design was transferred on to the PR using a lithography technique. 3) The circuit was patterned by wet etching of copper. 4) Through holes were made by a laser cutter. 5) Vias were made by filling the through holes with solder. 6) Solder paste was applied on the contact pads and the SCS chip, and other surface mount devices (SMDs) were populated on the pads. The

circuit was baked at 200 °C for 5 mins, and extra flux was applied for reflow soldering, if necessary. 7) Once all components were populated, a thick layer of epoxy (200 ~ 500 μm) was applied followed by 10- μm Parylene-C coating as a biocompatible package.

To evaluate the performance of epoxy-Parylene-C packages and the possible electrical failure of the chronic implant, active accelerated-lifetime soak testing was performed in saline at a higher-than- body temperature. For soak testing, a discrete version of the SCS circuit was designed using a MCU (MSP430, Texas Instruments, TX, USA), as shown in Fig. 7-10a. This circuit was programmed to mimic SCS stimulation patterns, once it received wireless power and data through the inductive link. Dimensions and fabrication process of the discrete circuit were identical to the SCS system. Four LEDs (LB QH9G, blue 466 nm, OSRAM, Germany) were integrated on the flexible PCB, and each LED was individually controlled by the MCU. Spring structured interconnects were utilized to reduce mechanical stress caused by stretching and twisting movements in the neck and shoulder area of the animal. A MEMS-based receiver (Rx) coil was fabricated separately to be placed on the back of the animal and connected to the SCS with flexible wires (392 F high-flex miniature wire, 36 AWG, McMaster-Carr. OH, USA).

Five discrete SCS devices were prepared and immersed in saline at 75 °C, as shown in Fig. 7-10b. Each was activated by coupling the Rx and Tx coils, and daily the samples were visually monitored for possible device failure. No delamination or major physical corrosion occurred after 14 days in accelerated lifetime testing conditions, while the long-term stability experiment is still ongoing. Using the Arrhenius relationship, preliminary results show that the implant lifetime can be equivalent to 3.5 years at body temperature of 37 °C, sufficient for one-year duration requirement in the animal study. Further improvements can be achieved by

optimizing temperature and duration of heat treatment for the Parylene/metal thin-film skin or by using additional chemical treatments.

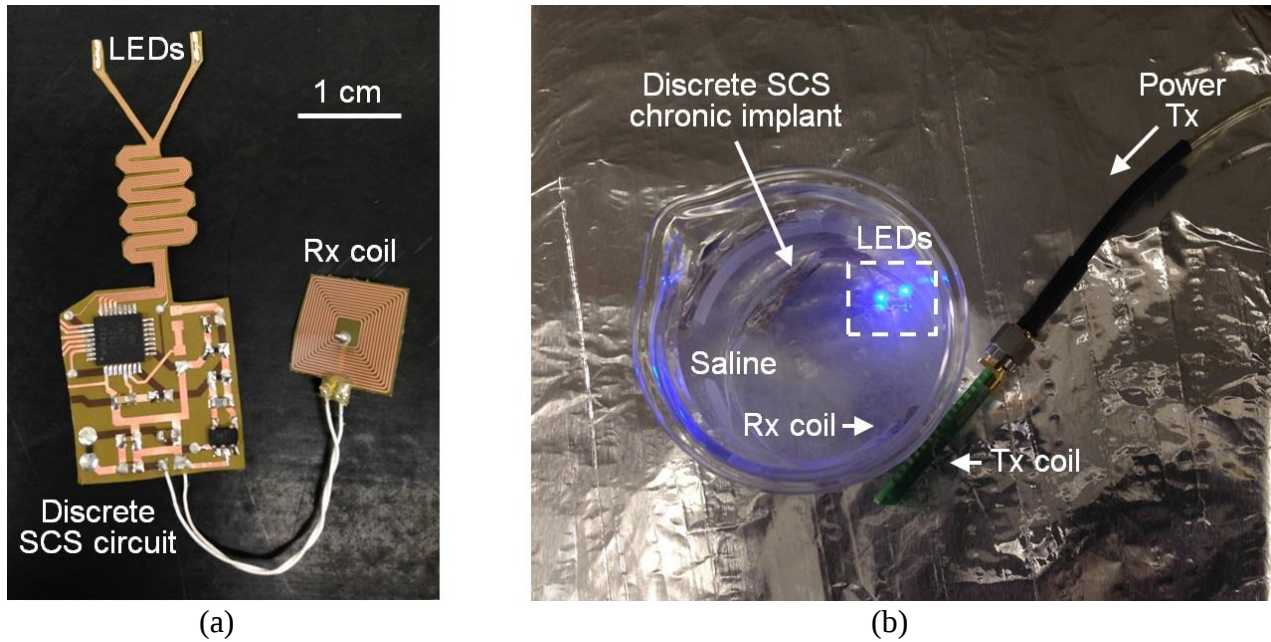


Figure 7-10. (a) Discrete SCS chronic implant and (b) accelerated soak testing in saline with wireless power and data transfer.

The discrete SCS system on the flexible circuit with the integrated LED array was chronically implanted to assess the feasibility and stability of the chronic SCS implant. The SCS board and Rx coil were coated with a thin layer of Polydimethylsiloxane (PDMS) to protect the surrounding tissue against damages caused by sharp edges. The Chr2 transduced rat was anesthetized and placed in a stereotaxic apparatus. Using sterile surgical procedures, a 2 ~ 3 cm incision was made in the skin overlying the skull, and the skin was detached from the skull using a scalpel. Once bregma and lambda sutures were exposed clearly, a hemostat was inserted under the skin of dorsal area, detaching the spinotrapezius muscle and skin to create a reservoir for the discrete SCS implant. The Rx coil was inserted at the center of the dorsal area through the channel, and the flexible circuit was placed around the rat's shoulder.

After both Rx coil and SCS circuit were in place, the Tx coil was inductively coupled with the Rx coil through the skin, and the discrete SCS system was tested to verify possible damage during insertion. Once functionality was confirmed, a small craniotomy was made to expose V1 using a micro drill (Ideal Micro-Drill™, Roboz Surgical Instrument Co., MD, USA). With the LED array in place, dental cement was applied to secure the array in position. A piece of gel foam was placed on top of the exposed V1 to cover the dental cement. The skin overlying the skull was sutured closed. After two days of recovery the rat was brought to the lab and slightly anesthetized with a mixture of oxygen and isoflurane. The discrete SCS implant was tested daily by visual inspection of the LED light through the skin. The implant was verified for 14 days, while no behavioral changes were observed.

7.5 Conclusion

Design and fabrication process of the wireless slanted optrode array for a really untethered bi-directional neural interface were presented. The array was inductively powered and controlled by the wireless SCS system, designed to improve power efficiency. The SCS system for implantable wireless optogenetics provides high instantaneous power through the inductive link to emit sufficient light and evoke neural activities. The LabVIEW PC interface and custom-designed power Tx module provide wireless power and data to the SCS system, while electrodes embedded in the optrode array enable simultaneous neural recording. The self-assembled LED array on a single substrate can reduce manufacturing cost. Acute *in vivo* experiments with optical stimulation and LFP recording have verified the efficacy of the SCS system for wireless optogenetics.

Chapter 8

Behavioral animal experiment

8.1 Motivation

As stated in Chapter 6, high risk is associated with development of new animal model studies, especially in behaving animal experiments, due to difficulty in interpreting stimuli-driven behavioral responses in animal models. For this reason, the concept of artificial sensory perception induced by optical neural stimulation based on optogenetics has been tested in a well-established behaving animal experiment to detect artificial auditory sensory perception, specifically tinnitus in mice model, prior to pursuing proposed behaving animal experiments in Chapter 6. A new chronically implantable wireless neuromodulator in behavioral mouse models has been developed to study whether optical modulation of specific subtypes of neural population can induce chronic tinnitus perception in freely behaving mice.

Chronic tinnitus is a prevalent auditory disorder that affects approximately 5-15% of the general adult population [182], [183]. An estimated 1-3% of those sufferers experience severe tinnitus that substantially reduces their quality of life, involving sleep disturbance, work impairment, and psychiatric distress [184]-[186]. Most cases of chronic tinnitus are associated with aging and early noise exposure [187]-[190], and at present are incurable [191]. Although tinnitus initially may be triggered by injuries to the auditory periphery, the neural generator of tinnitus most likely resides in CNS [192]-[197].

A variety of animal models have been explored in different species, where tinnitus is normally induced using either ototoxic drugs such as salicylate and quinine or exposure to loud

noise, and detected by either measuring animal behavior or imaging abnormal neural activity (in physiological models) [198], [199]. CBA/CaJ and C57BL/6J mice are well-established mouse strains to study noise-induced, or age-related hearing loss. They easily are modified by genetic methods, important for studying molecular pathways underlying hearing loss. This is another benefit of using a mouse model, since most drawbacks of a rat model in optogenetics study are the virus injection step. Extensive optogenetic mouse lines are based on these mice and, in the long run, the transgenic mouse line saves time and money.

Optical neural interface is an ideal solution to current limitations in behavioral mice. This neural interface should be applicable readily to other neurological disorders associated with dysfunctional neuronal plasticity. The novel, mass-producible, optogenetics tool for wireless optical neural modulation, would enable neuroscientists, clinical researchers, and the pharmaceutical industry to study neuronal mechanisms and behavioral readouts underlying CNS disorders.

8.2 A simple battery-powered wireless optical neuromodulator for freely behaving mice

The inductively powered wireless approach, described in the previous chapter, requires internal implantation of a receiving coil beneath the skin of the animals' back. The external transmitting coil must be aligned with the receiving coil that requires an external harness such as a backpack, and this approach is not feasible for small animals. To overcome this restriction, in addition to the inductively powered wireless system, this author has developed a simple, lightweight, and low-cost optical neuromodulator, which combines the flexible LED array with a battery-powered LED driver circuit, suitable for chronic implantation in freely behaving small animals such as mice. Depending on requirements of an experiment design, three LED controllers are available: a

magnetic switch controller, an infrared (IR) switch controller, and an IR-microcontroller (MCU) based controller.

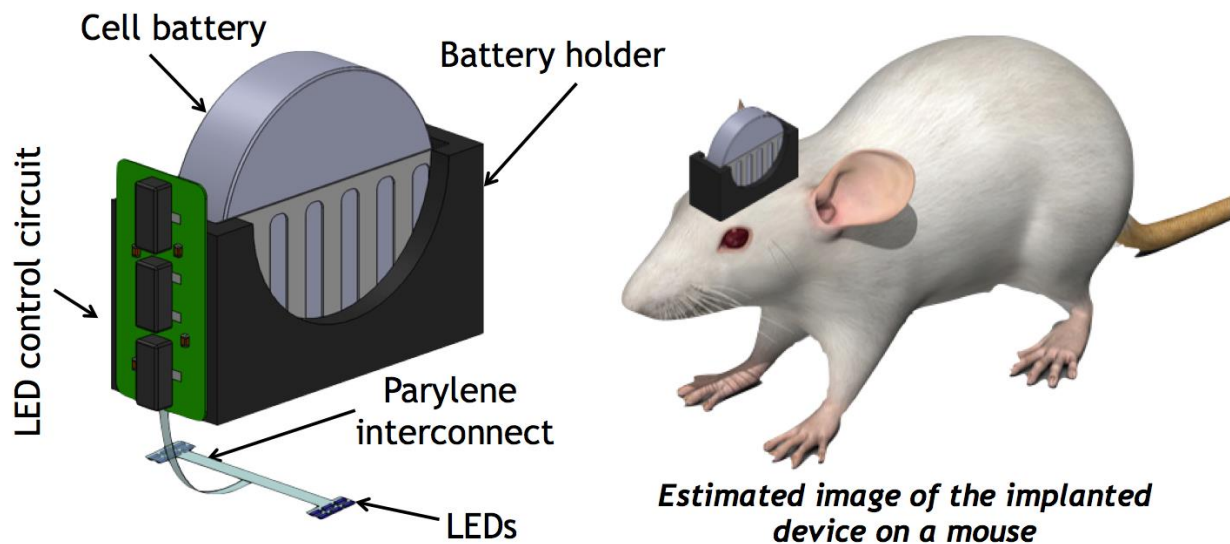


Figure 8-1. Basic design of battery-powered wireless optical neuromodulator

Basic design of the proposed battery-powered LEDs array comprises three major parts: battery holder, LED controller circuit, and LEDs array, as shown in Fig. 8-1. The battery holder (BS-1225-PC, MPD Inc., NY, USA) serves as a body as well as a power source. The LED controller circuits were attached on the wall of the battery holder to minimize overall size of the device, and the custom-designed LED driver was fabricated on a flexible double-layered polyimide substrate, Pylux®AP (AP7163E, DuPont, DE, USA), using UV lithography technique. Operation voltage is between 2.7 V and 3.2 V and it is supplied by a single BR-1225 lithium battery. This 3 V battery has nominal capacity of 48 mAh sufficient to drive two LEDs for approximately two hours without significant voltage drop. Each LED is connected to a current limiting resistor in series, and bypass-capacitors are connected to every semiconductor components. The battery-powered LEDs array can be integrated with a controller circuit or a flexible wire depending on location of implantation. The overall dimension of the device

including the battery is 5 mm × 12 mm × 6 mm and its total weight is less than 1.5 g which is less than 10% of the subject's body weight. The entire system comprises fewer than ten off-the-shelf components, and the assembly process requires no special equipment. Replaceable batteries extend the device's lifetime and eliminate any need for complex circuitry for inductive powering and/or battery recharging. The optically- transparent Parylene package ensures the device's safety and biocompatibility while maintaining its mechanical flexibility.

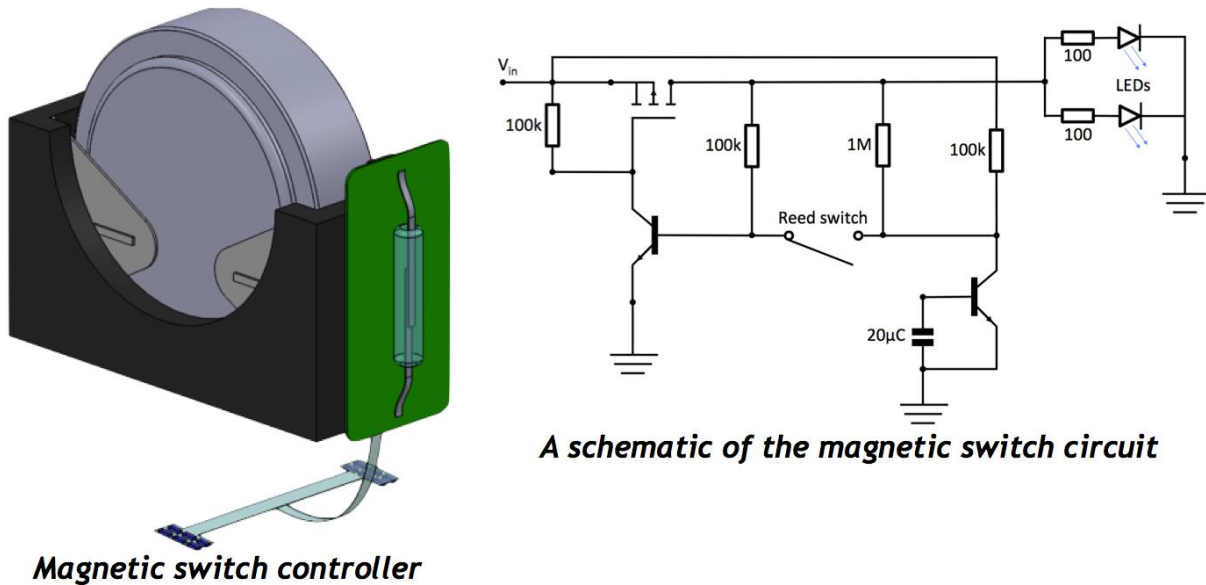


Figure 8-2. Design of the magnetic-switch based controller and a schematic of the LED driver circuit

8.2.1 Magnetic-switch based controller

Initially, the magnetic-switch based device was designed for a fully-implantable version of the device that requires an external switch to activate the implanted device. Basically, a latch circuit is connected to a capacitive oscillator through a momentary switch, in this case a reed switch. Once the switch is closed, the oscillator generates a pulse train with a specific frequency controlled by a capacitor value, and a latch circuit generates the same pulse train. When the switch is open, the latch switch stores a momentary state of the oscillator and stays. This allows

dual activation modes - oscillation mode and continuous activation mode. Attaching a small magnet on the reed switch activates oscillation with a fixed frequency. Removing the magnet from the switch triggers continuous activation or turning off the circuit, depending on the momentary state of the oscillation at the moment of the magnet removal. The latch circuit consists of a single bipolar junction transistor (BJT), single p-type MOSFET, and two resistors, and the oscillator comprises a single BJT transistor, two resistors, and one capacitor, thus the entire circuit requires only eight off-the-shelf components.

The magnetic-switch LED array is suitable for applications requiring a fixed-frequency modulation. If the experiment requires constant stimulation during an entire session, the system can be activated by attaching a small magnet on the circuit. Remote activation also is possible by applying a focused-magnetic field at designated locations, and this method is useful for an experiment designed to give a stimulus at specific locations and conditions.

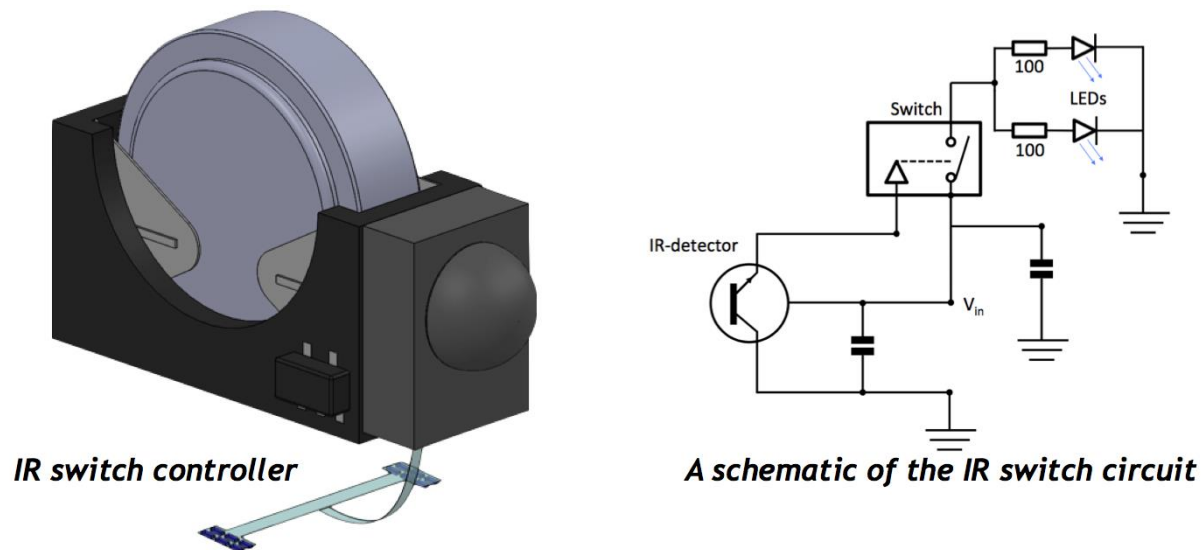


Figure 8-3. Design of the IR-switch controller and the schematic of the LED driver circuit

8.2.2 IR-switch based controller

In the IR-switch controller, the LEDs array is controlled wirelessly by a modulated IR signal generated by an external transmitter system. A digital oscillator with an external trigger is used

to generate alternating TTL signals at 38 kHz. The receiver unit is intended to be small to permit mounting on the skull of a freely-moving mouse. The IR-switch controller circuit comprises a low-power IR reception module (GP1UD271XK, Sharp, Japan) and an analog switch (ADG702BRTZ, Analog Devices, USA).

Main advantage of the IR-switch LED array is a variable modulation frequency. LED output frequency can be controlled externally by changing the TTL trigger of the transmitter unit. The receiver unit can receive the IR transmitter signal from relatively long distance (< 2 m) and a group of receiver units can be activated simultaneously.

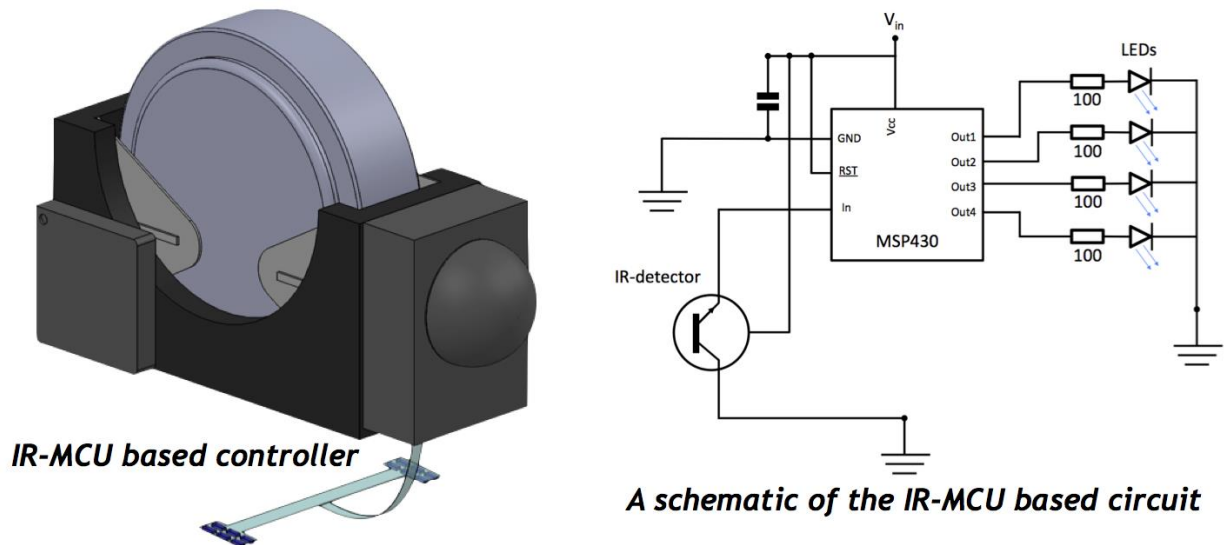


Figure 8-4. Design of IR-MCU based controller and the schematic of the LED driver circuit

8.2.3 IR-MCU based controller

The aforementioned two devices are capable of activating only a single LED or a group of LEDs at the same time. That limits spatial resolution of optical stimulation. IR-MCU based LED array is designed to activate individual LED units independently. A lower-power MCU (MSP430, Texas Instruments, Dallas, TX) controls activation of four individual LEDs, and the MCU receives an IR control signal from the external IR transmitter. A low-power IR reception module, directly connected to an input pin of the MCU, demodulates the signal from the IR transmitter,

and the MCU receives a pulse train. Depending upon a switch pressed, the transmitter generates a modulated signal with a specific number of pulses. Here the MCU works as frequency-counter as well as band pass filter to detect incoming frequency generated by the transmitter, and it performs a pre-programmed task based on the number of counts. The proposed IR-MCU based controller can control each LED independently with different frequencies, and pulse-width modulation (PWM) in MCU allows not only on-off states of LEDs, but also intensity control of LED output.

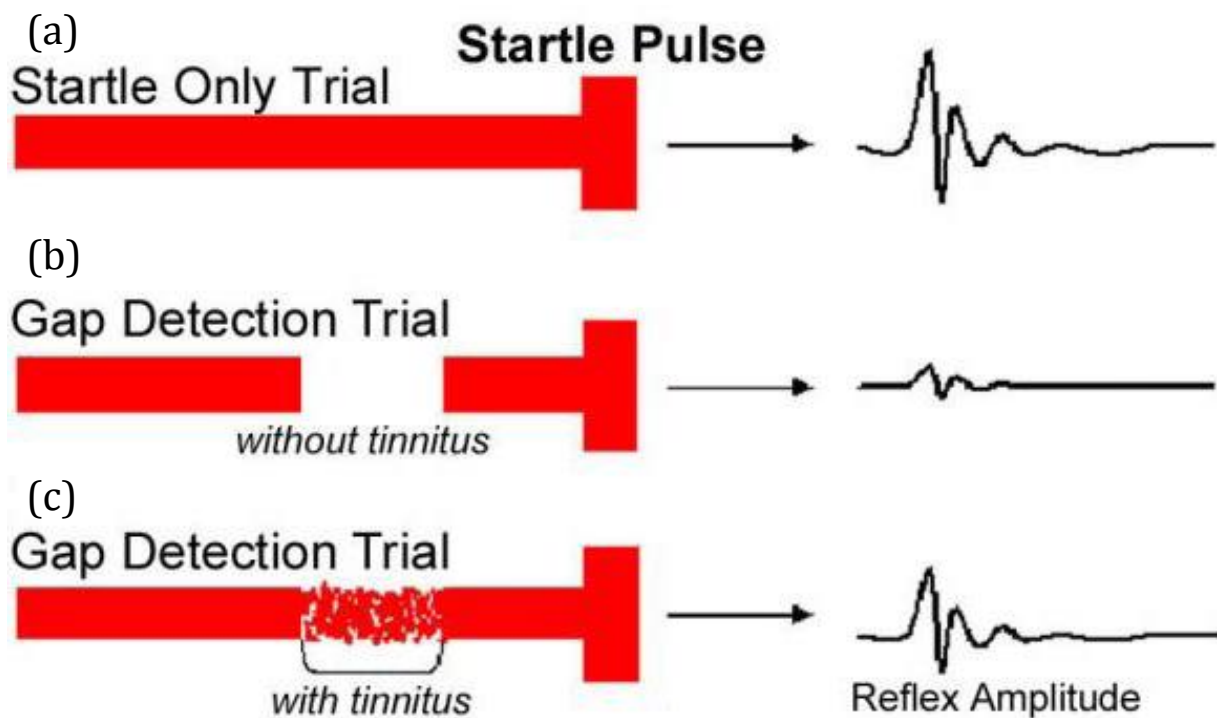


Figure 8-5. Summary of GPIAS for measuring tinnitus (adapted from [183]): (a) an animal is startled in the presence of a background noise plus a startle pulse. (b) A silent gap 100 ms before the startle pulse can reliably inhibit the reflex. (c) Due to tinnitus, the gap is not easily detected.

8.3 Behavioral experiment for tinnitus detection in a mouse model

Bao et al. have a long track record in developing genetically-modified animal models to study behavioral changes in mice. They used neuregulin-1 (NRG1), a molecule important for synaptic plasticity, to regulate the expression of neuronal neurotransmitter receptors and ion channels in

hippocampus [200]-[202]. A reflexive behavioral model, termed the gap-prepulse inhibition of the acoustic startle (GPIAS), was utilized to detect tinnitus in mice.

8.3.1 GPIAS method

The GPIAS method is based upon the ability of the acoustic startle reflex to be reduced by a preceding signal/stimulus, in this case a silent gap in an otherwise constant acoustic background. When an animal effectively detects such a silent gap, its response to a startle stimulus is reduced in magnitude (Fig. 8-5a and 8-5b). However, the presence of tinnitus would partially increase this reduction (Fig. 8-5c). The GPIAS method has proven efficient as a rapid screen for tinnitus in rats; requiring no prior training, no food or water restriction or aversive shock, and allows for many animals to be tested simultaneously in a relatively short (30 – 40 min) session [203]. Because there is no learned response to maintain, extinction is not an issue and the measure can be collected repeatedly across the lifespan, lending itself well to longitudinal studies for this project.

In mice, encouraging data have been obtained by the conventional GPIAS although additional testing is beneficial to eliminate both false positive and negative animals. The modified GPIAS method was designed to detect tinnitus induced by optogenetics neuromodulation. This new method can overcome both false positive and negative detection of tinnitus by the GPIAS alone, which also effectively overcomes the difficulty of determining the conscious perception of phantom sound in animals [203]-[205]. Therefore, the combination method to detect optogenetics stimulation-induced tinnitus in behavioral mice is designed.

8.3.2 The modified GPIAS method

The modified GPIAS method is a two-step test including the conventional GPIAS and the operational test. To determine whether optogenetics neuromodulation in the auditory cortex

induces tinnitus-like behavior, the GPIAS method is first used to detect the presence of optical stimulation-induced tinnitus among three groups of animals: Normal C57BL/6 control-, Auditory Cortical “Stimulation-On”-, and Auditory Cortical “Stimulation-Off”- groups. For animals in the “Stimulation-ON” group, their auditory cortex is stimulated optically with various light intensities of 1 – 10 mW/mm² and durations of 1 – 100 ms, using the implanted optrode interface. During the GPIAS testing, the animals’ behavioral patterns are monitored continuously to identify possible presence of tinnitus. As soon as a significant tinnitus-induced behavior is observed in the “Stimulation-ON” group, the cortical stimulation is turned off to study whether this light-induced tinnitus effect is reversible. The operational test (shuttle box) is used to further confirm the data. A conditioned place preference test is used to detect tinnitus-like behavior in mice. Test details are explained in Chapter 8.4.2.

8.4 Results

8.4.1 Characterization of the battery-powered wireless neuromodulator

Several experiments were conducted to characterize properties of the proposed neuromodulators. Devices were chronically implanted in a freely behaving mouse and behavioral tests were performed daily basis to demonstrate long-term stability and capability of the proposed devices.

Since these devices have a designated voltage source, it is important to ensure the array is capable of evoking neural activity. Irradiances of the battery-powered neural stimulators with fully charged battery was measured using an optical power meter (Model 815, Newport, CA, USA). As Fig. 8-6a shows, total irradiances of IR-switch and MCU based devices were in a similar range (4 – 6 mW/mm²), and these values were well above the minimum requirement for activation of ChR2 (1 – 5 mW/mm²). Although the magnetic-switch based device produced less optical output power than did others, that power was sufficient to evoke neural activity of

the ChR2 expressed neurons.

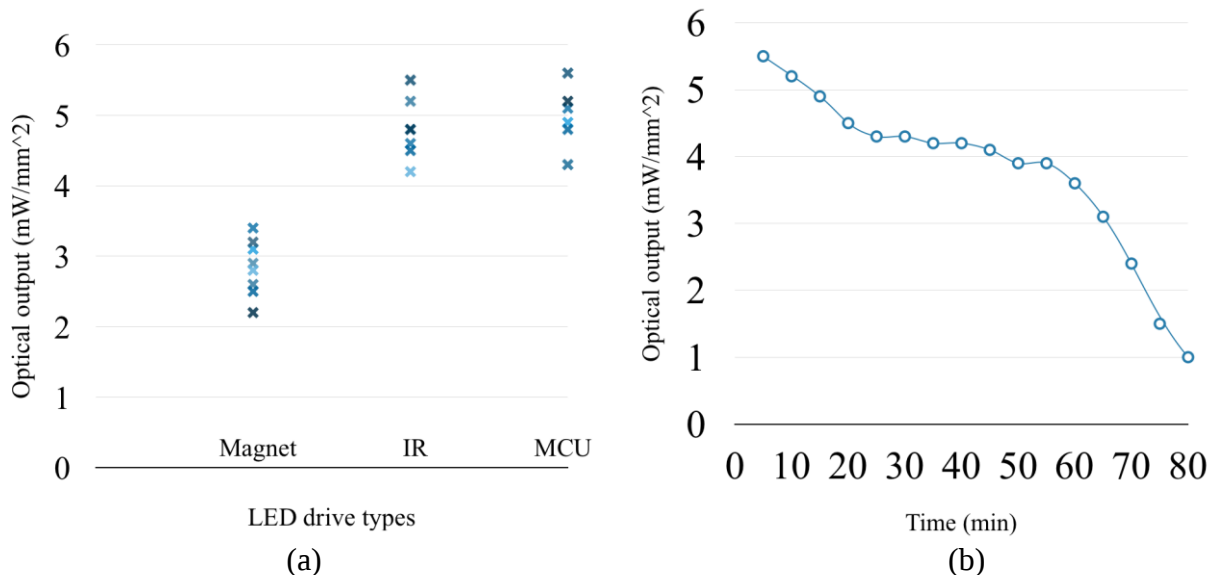


Figure 8-6. Optical characterizations of the battery-powered wireless optical neuromodulator.

The capacity of a single battery to maintain a certain light intensity (1 mW/mm²) was also measured. The IR-switch stimulator was activated at 50 Hz with duration of 10 ms for 5 hours continuously, and light intensity was measured every 10 mins. Slow exponential decaying of the LED light intensity was observed as shown in Fig. 8-6b. These results support the necessity of the replaceable battery for a long-term behaving animal experiment.

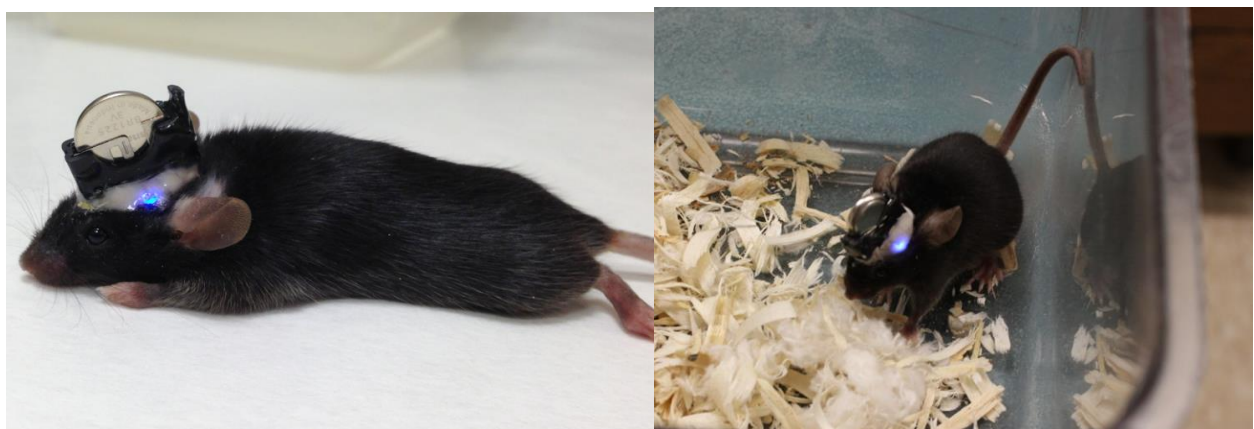


Figure 8-7. Chronic implantation of the LED controllers in freely-moving mice.

8.4.2 *In vivo* animal experiment

To evaluate safety and long-term reliability of the battery-powered wireless stimulator prior to the behaving animal experiment, devices were chronically implanted on the skulls of six mice (2 devices of each type). Details of the surgical procedure of the proposed *in vivo* chronic implant animal experiments are explained.

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University and Northeast Ohio Medical University. Transgenic mice (B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J) were used in this study. These transgenic mice express ChR2 fused to enhanced-yellow fluorescent protein (EYFP) under the control of the mouse thymus cell antigen 1 (Thy1) promoter. Mice underwent surgery to expose the dorsal surface of the auditory cortex. Prior to surgery, they were anesthetized with 4% isoflurane, then transferred to a small animal stereotaxic device. The hair over the dorsal surface of the skulls was removed (either clipped with scissors or by depilatory agent) and the skin was cleaned (alcohol and Betadine). Skulls then were exposed by a midline incision in the skin, the overlying muscles were reflected laterally and a small hole (<100 μ m diameter) made in the skull over the region of the auditory cortex. Incision length was approximately 20 mm, the minimum needed to attach the wireless device. The LED array was placed through the hole and into the auditory cortex, fixed to the overlying skull and cemented in place, closing the hole. Next, the battery holder was placed on top of the LED array and cemented to the skull using dental cement (3M ESPE Ketac Cem radiopaque, 3M, MN, USA). Topical lidocaine was applied to alleviate pain from surgical wounds. After surgery, the animals were placed in their cages to recover from the anesthetic. Once the animals awoke, they were housed back in the animal facility and allowed to recover for three days prior to behavioral testing.

After recovery the mice were brought to the lab and the implanted devices tested daily by activating the LEDs for 1 hour (Fig. 8-7). For more than two months, none of the six chronically implanted devices failed and the result demonstrates that the proposed battery-powered wireless neurostimulator is suitable for a long-term behaving animal experiment.

8.4.3 Behavioral animal experiment

The GPIAS test is similar to the previously-published method [183]. In short, the test was conducted with Kinder Scientific's GPIAS hardware and software (Kinder Scientific, CA, USA). Gap detection and prepulse inhibition (PPI) testing used background sounds presented through one speaker (Vifa XT25TG30-04) and startle stimuli presented through a second speaker (Powerline CTS KSN-1005) located in the ceiling of the testing chamber, 15 cm above the animal's head. The floor of the chamber was attached to a piezo transducer and provided a measure of startle force applied to the floor. A clear polycarbonate animal holder, with holes cut for sound passage, was suspended above the floor allowing the animal to freely turn around while minimizing excessive movement. The startle-eliciting stimulus was a 115-dB SPL, 20-ms duration broadband noise with a rapid rise gate (<0.1 -ms). PPI trials were periodically measured as a control condition. Gap detection trials are identical to PPI trials except for the inserted gap in the otherwise continuous background, as opposed to the inserted prepulse stimulus in the otherwise quiet background. Data from gap detection or PPI trials yield a single value expressed as a ratio of the mean response in gap trials/ mean response in startle-only trials. A response of 1.0 would mean that the startle reflex is the same whether or not preceded by a gap or prepulse stimulus (depending on the trial used). The lower the ratio is from 1.0, the better the startle inhibition. Individual and group data were first inspected for outliers, distributions, and descriptive statistics. Data were then subjected to analysis of variance (ANOVA) hypothesis

tests. All statistical analyses were conducted using Prism Graph Pad 5.0c (Graph Pad, CA, USA).

A conditioned place preference test was used to detect tinnitus-like behavior in mice. First, in a shuttle box, mice were trained to move into the illuminated compartment to avoid foot shocks when a sound is present, and stay in the dark side when it is silent. Among these trials, a total of 12 probe trials was randomly introduced in which no sound was played and no foot shock was delivered. Then, after the noise exposure, mice underwent the same test. Development of tinnitus-like behavior was detected based on mice preference for the illuminated compartment in the probe trials.

8.5 Conclusion

As a part of development of a cortically visual prosthesis based on optogenetics, the concept of artificial sensory perception induced by optical neural stimulation was tested in the GPIAS test for tinnitus detection in mice model. Three types of chronic wireless neuromodulators were developed to adapt the hybrid optical neural interfaces to a chronically implantable device on behaving small animals, specifically in a mouse model. Each device was designed to serve different applications, and the *in vivo* chronic animal experiment result proved that the proposed battery-powered wireless neuromodulators are suitable for long-term freely behaving animal experiment. The modified GPIAS test was proposed to overcome the limitation of the conventional GPIAS test alone for tinnitus detection in mice model, and currently the evaluation of the proposed method is in progress.

Chapter 9

Conclusion

Electrical neural stimulation has been a powerful tool for decades in electrophysiology and clinical neuroscience. However, its limitations necessitate improved technology. Recent developments in optogenetics have demonstrated the ability to target specific types of neurons with sub-millisecond temporal precision via direct optical stimulation of genetically modified neurons in the brain. Optogenetics has become a popular technique that has not only replaced electrical neuromodulation, but also opened a new field in the study of the nervous systems. The success of optogenetics has also opened new research area including the development of new light-sensitive proteins and technologies for light delivery.

In this dissertation, a series of hybrid neural interfaces, combining optogenetics-based optical neuromodulation and electrical neural recordings, have been designed, fabricated, and characterized to demonstrate capability of simultaneous optical stimulation and electrophysiological recording of cortical neurons. The proposed hybrid neural interfaces allow the possibility of achieving a truly untethered system with integrated multi- μ LEDs array crucial for chronic implants. 3-D light distribution using the 3-D waveguide array significantly improves the spatial resolution of light delivery.

The development of the hybrid neural interfaces has led an invention of several enabling technologies. Self-assembly of μ -LEDs using a PDMS stamp and low melting point solder enables a large population of micro-scale chip assembly on flexible polymer substrate possible. The DBE method for making slanted microneedles was designed, developed, and characterized. The results have shown that the three key parameters of the microneedle (the length, tip and base

diameters) can be controlled precisely by manipulating the droplet volume and the mask design (the aperture diameter and the distance from the center). The measurements of these parameters were consistent with the analytical estimation, demonstrating the repeatability and controllability of the DBE method for fabricating varying-length microneedles.

Based on works presented above, this author aims to develop a cortically based opto-epidural visual prosthesis for therapeutic management of all forms of blindness. The hypothesis that epidural optical stimulation of a large population of ChR2 expressing neurons in the primary visual cortex (V1) can induce phosphenes was formulated based on the studies of electrically-based cortical visual prosthesis. As a proof-of-concept study, three major tasks have been identified including stimulus-driven ECoG data analysis, a chronic device development for freely behaving animal experiment, and a behavioral animal test to examine the ability of optical stimulation of ChR2 expressing neurons to induce phosphenes.

First, stimulus-driven ECoG data have been analyzed to study the influence of cortically optical stimulation on a large population of neurons in rat V1. Preliminary results demonstrated reliable and consistent phase-locked synchrony on both the visual and cortically optical stimulations. Differences in phase modulations between the natural stimulus driven neural oscillations and the optically stimulated neural oscillations were also studied, in terms of latency and specific frequency bands of phase-locked synchrony. More stimulation parameters, such as duration, light intensity, and size of stimulation site, must be explored to improve the Opto- μ ECoG system, to replace a natural sensory feedback system.

Second, a chronic device for freely behaving animal experiment has been developed based on inductive link-based powering and data transfer technology. The wireless slanted optrode array for a really untethered bi-directional neural interface has been developed, and the array was

inductively powered and controlled by the wireless SCS system. Acute *in vivo* experiments with optical stimulation and LFP recording have verified the efficacy of the SCS system for wireless optogenetics. Moreover, the hermetic sealing method for chronic implantation is under development to enable implantable optogenetics with the SCS system.

Finally, the concept of artificial sensory perception induced by optical neural stimulation based on optogenetics has been tested in a well-established behavioral animal experiment to detect artificial auditory sensory perception prior to pursuing proposed behaving animal experiments. A new chronically implantable wireless neuromodulator in behavioral mouse models has been developed to induce chronic tinnitus perception in freely behaving mice.

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