High-density Optrodes for Multi-scale Electrophysiology and Optogenetic Stimulation

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***Abstract*— We demonstrate the design and implementation of hybrid optical-electrical probes ('optrodes') for high resolution electrophysiology and optogenetic stimulation of neurons in multiple brain areas. Our 64-channel implantable optrodes are minimally invasive (50 μm × 20 μm) and span 1~2 mm. To minimize tethering forces on the brain tissue a monolithic high-density flexible cable (6 μm thin) connects the probe to a lightweight headstage (1.3 gr, 256 channel configuration) designed for awake, freely-behaving small animals. A polymer-based multi-channel photonic light delivery system is integrated on shank in a separate layer, providing local optogenetic stimulation of the neural population adjacent to the probe. The entire manufacturing process, including the nanofabrication of the optrodes, post-fabrication assembly, and surgical implantation procedures are designed to be scalable, high-yield, and high-throughput.**

INTRODUCTION

A multi-scale, mechanistic understanding of brain function that includes both local- and whole-brain circuits remains an elusive goal of systems neuroscience. One of the fundamental difficulties in such studies is the lack of tools for high-resolution monitoring of local neuron ensembles simultaneously in different regions of the brain in awake, freely-behaving animals. Several longstanding hypotheses – including long range communication through coherence, mechanisms of multisensory integration, and the behavioral significance of cell assembles – could be tested if there were tools for distributed recording and stimulation that retain single-cell resolution. Given the ample and growing evidence that many cognitive behaviors involve neural circuits distributed across multiple brain areas [1], [2], the value of such an instrument to brain science would be significant.

Despite recent advances in neural recording and stimulation, including a rapidly expanding optogenetic toolset [3]–[6] and multichannel electrode arrays [7]–[11], we still lack a robust, high-density, low-cost, and most importantly *scalable* electrophysiological recording and optogenetic stimulation platform [12]. The ability to independently deliver light to multiple, highly localized (~50μm^3) regions of the brain would revolutionize in-vivo optogenetic experiments. A major challenge is delivering light to a subset of cells that have expressed opsins [13]. Illuminating the whole brain or a large volume of it using external fiber-coupled light sources does not provide the requisite spatial resolution and since the intensity falls off rapidly only a small fraction of target neurons in the vicinity of the light source (~100 μm) can be excited. To address this issue, high power light sources are currently used; these result in the generation of excessive heat in the brain, and potential for phototoxicity [14]. Based on the Kubelka–Munk model, as the light travels in the tissue it undergoes scattering, absorption, as well as Gaussian diffraction spreading [15], and light intensity is reduced and rapidly falls below the excitation threshold of opsins. Therefore, there is a trade-off between the range of stimulation and the required optical power, resulting in an inherently low spatial resolution [14], [16], [17]. Given the large size of conventionally-used fiber optics (usually 10-200 μm in diameter) and strong scattering of the light beam in the brain, only one or a few of them can be used in optogenetic experiments [18]. In view of these limitations, a number of recent studies have investigated the possibility of using optical light guides to deliver light to target locations below the surface of the brain, for example, by having fiber optic light guides straddle the silicon probe shank [18], [19]. The prohibitive size of fiber optics (e.g., 200 μm) limits the number of possible light delivery channels to one per shank, and increasing the number of channels makes the overall size of the probe too large, causing a large amount of tissue displacement during insertion. Cumbersome, labor-intensive manual assembly is another drawback of this approach. An alternative is to directly embed light sources (lasers or LEDs) on the probe shanks themselves [20], [21]; this results in excessive heat generation due to the inefficiency of these light sources and can potentially be hazardous if the toxic active elements (laser gain material) becomes exposed in the brain. On balance, it is desirable to keep the light sources outside and guide light to the target locations inside the brain; the problem then is one of routing many channels. Recently, compact photonic waveguides have been proposed that can be realized on a rigid dielectric substrate [5], [13], [22]. These photonic-only probes, while still undesirably large, have shown promise for providing multiple light delivery channels on one or more silicon shanks. These waveguides are rigid and their connection to the light sources outside the brain result in high tethering force on the brain tissue. Another major challenge is efficient coupling of light (in and out) of these optical in a controlled manner. Recently proposed light-coupling schemes based on a scanning galvanometer or a DMD chipset [5] are not suitable

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for chronic measurements in awake, freely behaving animals.

In this paper, we describe the design and development of an optrode in which microfabricated photonic polymer optical waveguides are monolithically integrated with hybrid silicon-parylene electrical probes to record and stimulate multiple locations in the brain with single cell resolution.

I. Optrode Structure

Our optrodes consist of an electrical layer and a photonic layer (Fig. 1). The electrical layer is designed to record extracellular action potentials from hundreds of neurons in the vicinity of the probe shank spanning 1~2 mm (depending on the recording site layout) with excellent unit isolation. The photonic layer is designed to deliver localized light from external light sources to different locations along the shank.

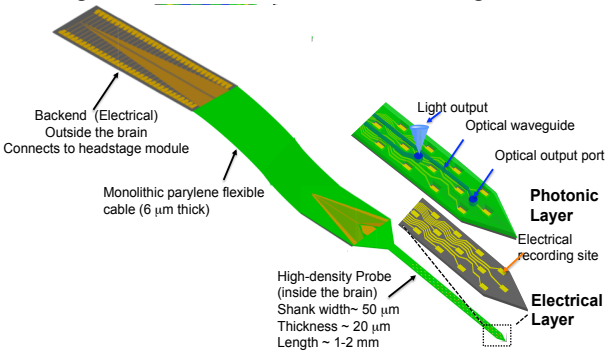

Figure 1. 3D schematic of the optrodes comprising an implantable Si probe, a flexible cable, a photonic light delivery layer, and a Si backend.

A. Electrical Recording Layer

The electrical layer consists of recording sites distributed along the probe shank connected to a headstage recording module through high-density interconnects. High-resolution (250 nm) electrical interconnects were realized to minimize the shank width. The 64-channel probes have a very small cross-sectional footprint (width less than 50 μm and thickness ~20 μm). The electrical interconnects expand on the parylene cable and extend the electrical connections to a silicon backend that interfaces with an adaptor printed circuit board (PCB) of the headstage recording module (Fig. 2).

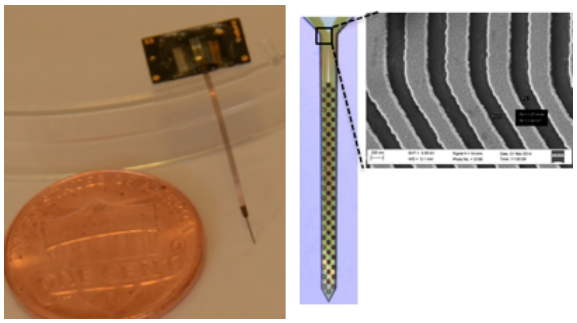

Figure 2. Left: A probe monolithically integrated with a parylene flexible cable, assembled to a recording headstage adaptor PCB. Right: Microscope image of the implantable part of the probe, with recording sites spaced 28μm apart in a hexagonal array. Inset shows the scanning electron micrograph (SEM) of the high-density electrical interconnects.

Existing planar probes can only record neurons on the same side as the recording sites because the silicon shank insulates the spike field potentials of neurons on the backside. Double-sided probes have been made [23], but require photolithography on both sides of a very fragile 20 μm thin wafer, making handling difficult, and rendering the whole process expensive and inherently low yield. A novel aspect of our optrodes is the capability of recording from both sides of the probe without the need to process both sides. Our solution is to etch front-to-back vias that are later 'plugged' with electroplated conductive materials (e.g. poly(3,4-ethylenedioxythiophene), PEDOT or carbon nanotubes) to effectively double the recordable volume without increasing the volume of tissue displacement (Fig. 3).

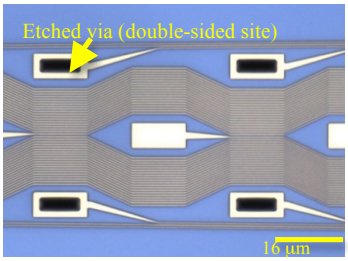

Figure 3. Single and double sided recording sites.

B. Flexible Cables

Flexible cables made of parylene polymer are monolithically integrated with the probe to provide a compliant connection between the implanted silicon probe and the electronic headstage modules outside the skull (Fig. 2). The diminutive size of these 64 channel flexible cables, 20 mm long, 0.5 mm wide (with an 8 μm wire pitch), and 6 μm thin enables multiple probes to be implanted within the confined area of the skull even in small rodents such as mice. These cables are made using a well-established process [24], which we previously developed for a variety of implantable devices such as micro-ECoG arrays [25]. The fabrication process is scalable by adding additional layers to increase the channel capacity without increasing the lateral dimensions of the cable. Our parylene cables are far more flexible and smaller than their commercial counterparts, and are small enough to fit inside narrow gauge cannulas for targeting deep sub-cortical brain structures in larger animal models such as cats and non-human primates. The comparison of our flexible cable with the state of the art (all with 64 channels and a length of 30 mm) is given in Table I.

Comparing the beam stiffness of these cables shows that our parylene flex cables are almost 1000 times more compliant than the best available neural flex cables [26] and 5,500 times more compliant than the best standard commercially available flex cables [27] both made of polyimide.

TABLE I. Comparison of our Flexible Cables with the State of the Art

64-channel Cables Length ~ 30 mm	Shear Modulus (GPa)	Pitch (μm)	Width (mm)	Thickness (μm)	Stiffness (N/m)
Commercial Neural Felx Cable (NeuroNexus)[26]	2.8	75	4.8	20	2.7×10^{-3}
Commercial Flex Cable (MaxiFlex)[27]	~ 3	76 (50)	5	100-400	1.4×10^{-2}
This work	0.98	4	0.42	6	2.5×10^{-6}

Conventional 'flexible' cables, like those used in mobile cellular phones, are comparatively very stiff and large, nor are they readily amenable to integration with microfabricated structures (Table I). Also, the size and pitch of our cables are at least one order of magnitude smaller.

C. Photonic Layer

The photonic layer consists of an array of polymer waveguides embedded in parylene. As shown in Fig. 1, each waveguide terminates every ~75 µm along the probe shank to illuminate the local volume next to the waveguide output port. The waveguide termination is designed in a way that light is deflected normal to the surface of the optrode so that the same neurons monitored by the electrical recording sites can be stimulated using optogenetics. Each optical waveguide is designed to support a fundamental guided photonic mode, even for extremely small dimensions. For example, the guided mode profile of the prototype waveguide (cross sectional dimensions of 2 µm × 2 µm) is shown in Fig. 4. These waveguides are flexible and can be routed through flexible cables to external laser sources outside the skull.

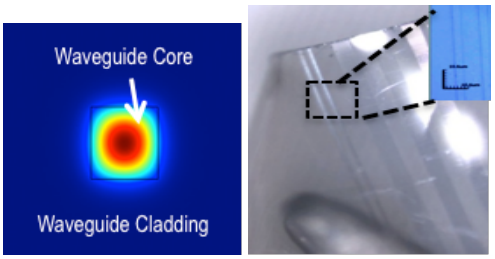

Figure 4. Left: Mode profile of a single photonic waveguide. Right: An array of photonic waveguides on a flexible parylene substrate.

II. Headstage Module

In collaboration with e3 Neurotechnology LLC, we have designed a radically compact and light-weight neural recording system (Fig. 5) exploiting low power, caseless custom ASICs built into the headstage [28]. High-density, 0.8 mm profile board-board connectors allow up to ten 64-channel modules to be stacked on top of each other without increasing the footprint on the animal's head (Fig. 5). The overall dimensions of each module, including connectors, is 9 x 9 x 2 mm; it weighs 250 mg and interfaces with a 64-channel silicon probe via the flex cable. Power, ground, and digital control signals for each ASIC are shared across modules. With this approach, 256 recording channels occupy only 1 cm^3 with a total weight of 1.3g (5% of the weight of a normal adult mouse). At the heart of each module is a fully integrated, low-power, low-noise amplifier array (RHA2164, Intan Technologies LLC, Los Angeles, CA). This single-chip ASIC contains 64 amplifiers with programmable filter bandwidths suitable for recording single unit, LFP, and EEG signals. Low input-referred noise levels (1.7µV rms) enable reliable detection of extracellular spike signals as small as ~25µVpp. The upper bandwidth of the amplifiers may be programmed between 10Hz and 20kHz, and the lower bandwidth from 0.02Hz to 1.0kHz. Each amplifier has a 3rd-order Butterworth low-pass filter to reject non-neural signals and noise beyond the desired bandwidth, and to minimize aliasing. Internal capacitors reject any DC offset voltages at the input pins, eliminating amplifier saturation due to junction potentials at the electrode-tissue interface. Additional on-chip circuitry provides user-selectable access to any of the 64 amplifier input pins to electroplate or activate electrode sites prior to implantation, or for *in situ* electrode impedance testing. A dual high-speed, 14-bit ADC (AD7946, Analog Devices) with a single serial digital output stream samples 64 channels at up to 32kSamples/s per channel.

A microcontroller module at the top of the stack provides power conditioning, a common high-speed clock, digital lines for mode selection, and additional data multiplexing (10:1) using a high-speed serializer chip (SN65LV1023A, Texas Instruments). The headstage connects to a 6 wire cable (1.0mm O.D) to relay power, control signals, and high bandwidth LVDS data stream to the data acquisition PC.

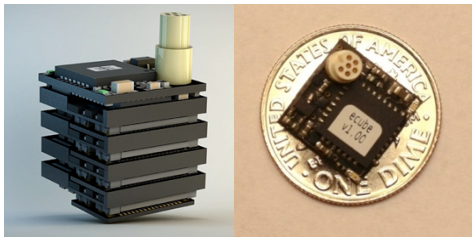

Figure 5. Left: Stackable headstage, 256 configuration. Right: 640 channel headstage shown on a dime for scale.

III. Optrode Characterization and Implantation

Post-release from the wafer the electrical recording sites are modified through electroplating conductive polymers (PEDOT: PSS). PEDOT reduces the recording site impedance by at least one order of magnitude (Fig. 6), thus increasing the signal-to-noise ratio (SNR). The headstage stack is assembled and fixed to the skull and each probe is independently implanted into the cortex using a robot-assisted stereotaxic micromanipulator (Neurostar, Germany) equipped with piezo-actuated micro-tweezers (Smarac, Germany) (Fig. 7). This tool allows probes to be inserted into the brain without exerting lateral force that may damage the brain tissue.

IV. Conclusion

We have demonstrated a scalable process for fabricating compact, high-density optrodes for electrophysiology recording and optical stimulation in multiple brain areas. We have optimized the manufacturing process so that many of these probes (1000's) can be fabricated in each fabrication run. We have also implemented a lightweight, scalable headstage module that can support multiple independent, implantable optrodes. In the presentation we will discuss further details of the backend integration with laser sources and electronic control module. We will also present preliminary data from our acute electrophysiology recordings.

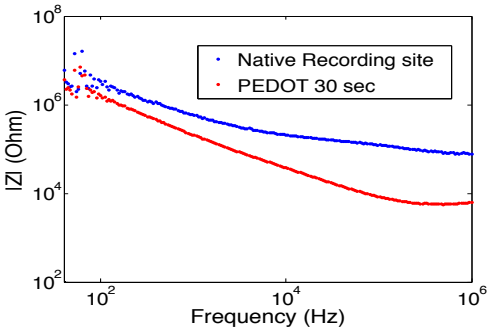

Figure 6. Impedance spectroscopy in saline solution before and after electroplating conductive polymer PEDOT.

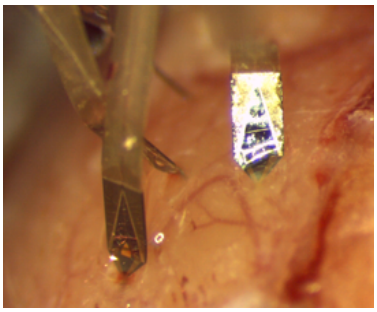

Figure 7. Four independent probes implanted in a the mouse visual cortex.

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