Fabrication of neural probes for simultaneous *in vivo* optical stimulation and electrical recording in the brain

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The function of a cortical neuron depends on its microcircuitry – the inputs it receives from local and long-range connections and the outputs it sends to other neurons.[1] Optogenetics,[2,3] uses genetic manipulations to insert opsin containing ion channels into cells. Then light can be used to optically gate ion-transport across the plasma membrane to control spiking activity with millisecond precision. Coupled with virus-mediated RNA transfer, this capability enables the determination of neural circuits with greater cellular specificity and spatio-temporal resolution than previously possible.

Several technologies have been reported for fabricating *optrodes*, neural probes with an optical channel to excite or inhibit the activity of targeted neurons and electrode arrays for localizing and mapping neuronal dipoles in the target region. An advanced probe architecture has recently be proposed.[4,5] Shown schematically in Fig. 1, it is comprised of at least 6 tiers of 4-channel sensors spaced about 10 μm apart beginning at the tip of the probe. An optical fiber substrate provides efficient light delivery and synchronous spikes mapping requires electrode site diameter less than 5 μm.[6] This precision and level of integration on a non-planar surface, are far beyond any approach reported to date.

A 2-channel prototype has been fabricated by ion beam proximity lithography with plasma deposited resist.[7] The stencil mask consists of a family of 50 intersecting V-grooves anisotropically etched from opposite sides of a (100) silicon wafer. Fiber substrates are held in grooves on one side with very light springs and the resist exposed from the opposite side by a beam of He^+ ions (Fig. 2). This strategy bypasses the very difficult problem of on-fiber alignment over ~5 cm lengths. Figs. 3 a) and b) show *in vivo* recordings taken with the prototype in a prosimian (bush baby) and a mouse. We will report an extension of this fabrication concept to the advanced, multi-tiered probes described above at the conference.

[1] R. Douglas and K. Martin, *Annual Review of Neuroscience* **27**, 419-451 (2004).
[2] K. Deisseroth, *Nat Methods* **8,** 1, 26-29 (2011).
[3] F. Zhang *et al*., *Nat Protoc* **5**, 439-456 (2010).
[4] F. Mechler *et al*., *J. Neurophysiol* **106**, 828-848 (2011).
[5] F. Mechler and J. Victor, *Journal of Computational Neuroscience* **32**, 73-100 (2012).
[6] D. Hill, S. Mehta, D. Kleinfeld, *The Journal of Neuroscience* **31**, 24, 8699-8705 (2011)
[7] D. Parikh et al., *Journ. Microelectromechanical Systems* **17**, 735-740, (2008).

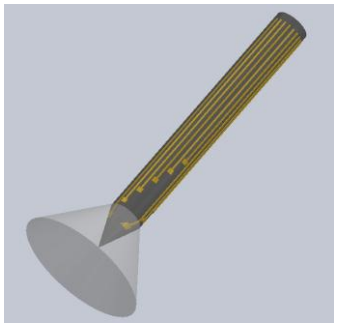


Figure 1: Integrated thin-film optrode with 6 tiers of 4-channel (tetrode) sensors on an optical fiber substrate.

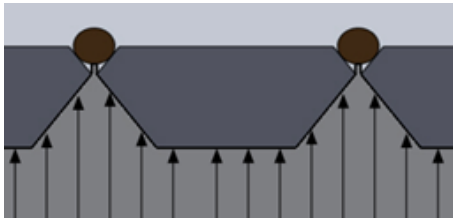
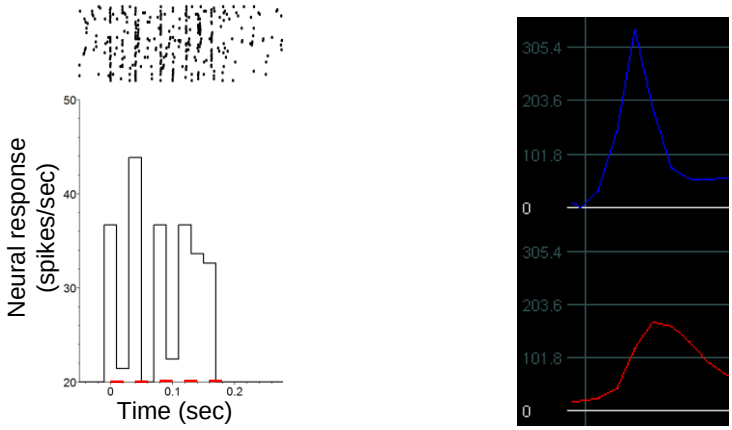


Figure 2: Mask-to-fiber alignment in 2-channel prototypes is accomplished by a micromachined silicon jig. The jig is formed by two families of intersecting V-grooves, anisotropically etched [Nanostructures Inc.] into opposite sides of a (100) silicon wafer. The depth of the upper grooves is chosen so that the fiber extends a small distance above the plane of the wafer. The depth of the lower grooves determines the width of the rectangular opening between the top and bottom grooves. Exposing the jig with energetic (30 keV) helium ions, as shown, forms a negative tone image of the mask openings on the fibers. Electrodes are printed around the circumference of the fiber in a step-wise fashion. Sputter etching is used to transfer the resist pattern to thin films on the fiber.



a) b)

Figure 3: a) In vivo testing in adult male prosimian primates (Otolemur garnettii). Five laser pulses were delivered, each 25 msec duration with a 25 msec inter-pulse-interval (red lines on the time axis). Neural signals were amplified and band-pass filtered at 250Hz-7.5kHz to remove any photoelectric artifacts. The filtered signals were thresholded at 3.25 times the r.m.s value to extract spikes. Spikes were sorted online using clustering algorithms on the Cerebus suite to extract a single unit. In layer 5, we found neurons that systematically fired spikes in response to each 25 msec laser pulse. The spikes were time-locked to the laser onset; b) A single spike recorded in mouse hippocampus shows different spike amplitudes on the two probe channels, a primitive demonstration of source localization.