Adaptive Fluidic Microoptics for Single Cell Optogenetics*

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Abstract: We present an electrically controlled, micron-scale liquid lens that provides adjustable focusing and beam steering, which is being developed for optogenetic *in vivo* mapping of brain activity with single cell resolution.

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1. Introduction

In the last decade, optogenetics has emerged as a powerful tool to monitor and control the functioning of populations of neurons *in vivo* [1]. Optogenetics consists of the selective photoexcitation of neurons, genetically modified to express photosensitive membrane proteins (opsins) [2]. Upon excitation, these opsins react by transporting ions into or out of neurons to control their electrical activity. So far, most optogenetics studies have involved photoexcitation with limited spatial resolution, relying on orthogonal genetically-modified channelrhodopsins that respond to different colors of light that are selectively expressed in different classes of neurons [3]. Two-photon control is possible, but only within the shallow depths (e.g. <1 mm) afforded by two-photon penetration into the brain [4-6]. Thus, it is highly desirable to have a method of exciting individual neurons at arbitrary sites in the brain in a controllable and single-cell manner, as this would open the possibility of *in vivo* analysis of network connectivity at the single-cell level, even in difficult-to-access deep brain tissue. Our ultimate goal is to develop an implantable optical probe that has active focusing and steering optics placed at the end of the probe that will enable light delivery from an external laser to individual neurons. Here we discuss progress that we have made in the first phase of our work, namely engineering micron-scale liquid lenses with active electronics to enable both focusing and steering. These lenses are designed so that in later phases of the program they will be integrated to waveguides operating in the red/near-infrared, building from previous work we have done on microfabricated waveguide-mediated optogenetic control [7], thus enabling two-photon optogenetic excitation of individual cells at arbitrary locations in living mammalian brains.

2. Adaptive Liquid Microlenses

We employ optofluidics, whereby microlenses comprised of liquid with diameters (D_o) as small as 50 μm are formed by shaping the interface between two immiscible liquids with different refractive indexes. Electrowetting [8] is used to control the shape of the liquid-liquid interface, thus providing variable focus and beam steering functionalities. In electrowetting, the surface energy of the solid substrate is modified by the application of a voltage, changing it from hydrophobic to hydrophilic in prescribed regions via suitably designed electrodes covered by a hydrophobic film. Previously, liquid, plano-convex, adjustable focal length microlenses with electrode diameters as small as 200 μm were demonstrated [9]. In the current work, beam steering and significant size reduction were introduced via a novel design of the substrate and electrodes.

A target volume containing ~100,000 neuron cells bodies translates to a microlens with an adjustable focal length of 0.1 – 1 mm with simultaneous steering over ±5° capable of delivering light to a spot size of ~10 μm. To minimize the overall size of the optic both focusing and steering adjustments are made via a single optical element. Both functionalities are achieved by embedding the liquid interface in a conical taper, etched into a fused silica substrate, which has interdigitated electrodes along the sidewall as shown in Figure 1. Initial designs employ four independent electrodes or a single electrode around the conical taper. One of the liquids needs be conductive, typically water, and the other phase, an insulator, typically a non-polar solvent like oil for electrowetting to work. The positioning of the oil and water forms of the microlens and is controlled by a patterned hydrophobic film over the electrodes and a surrounding hydrophilic surface. Oil resides inside the taper over the patterned hydrophobic film. When a voltage is applied the surface energy changes from hydrophobic to more hydrophilic, allowing water to wet over the electrode. This changes the radius of curvature of the liquid interface and thus the focal length of the microlens.

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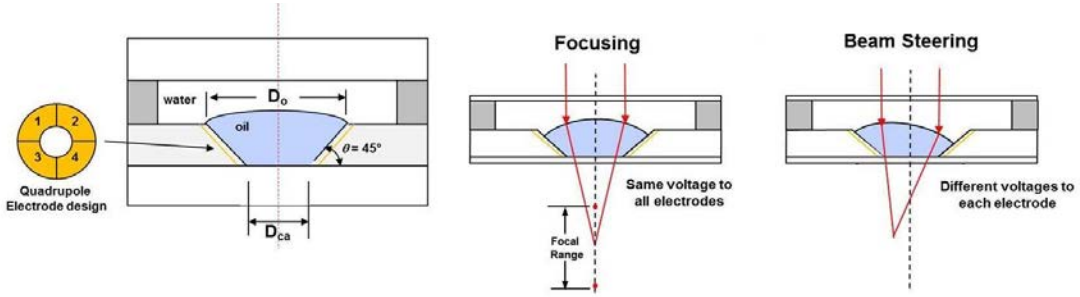

Fig. 1. Liquid microlens design that combines both active focusing and steering by controlling the interface formed between two immiscible liquids. The liquid interface is contained within a 45° conical taper that has a series of patterned metal electrodes along the sidewall. Through electrowetting, the interface curvature changes as a function of applied voltage to the electrodes. Variable focusing along the optical path will occur when the same potential is applied to all the electrodes. Beam steering will occur when different potentials are applied to each electrode.

The fabricated microlenses have a 12 µm deep, conical tapered cavity with a 45° sloping sidewall that is etched into a 750 µm fused silica wafer using a gray-scale lithography technique [10]. A special optical mask was designed that had sub-resolution sized features which created a tapered profile in photoresist. The tapered pattern was then transferred into the fused silica using an optimized RIE process. Next, 250 nm of aluminum was deposited and etched to form the electrodes. Some microlenses had a quadrupole arrangement and in others just a single circular electrode was used. For the single circular electrode design, only adjustable focusing can be achieved. After the electrodes are patterned, 500 nm of PECVD oxide is deposited, forming the dielectric layer. The final fabrication step is to create the hydrophobic/hydrophilic regions. We use an 80-nm-thick CYTOP (Asahi Glass) as the hydrophobic film. The film is then patterned and etched in oxygen plasma to form hydrophobic regions inside the taper over the electrodes, leaving hydrophilic regions elsewhere. Figure 2 shows several images from the microfabrication process.

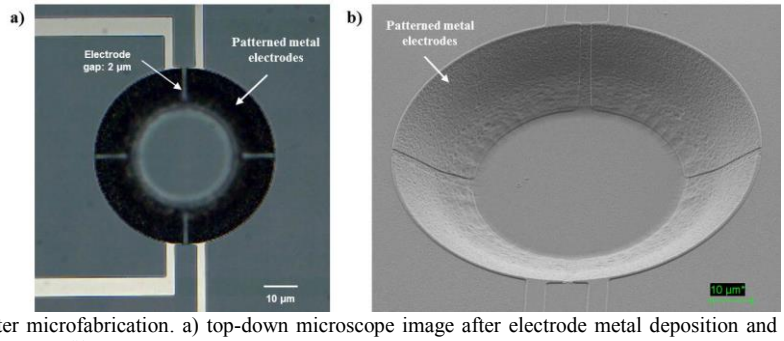

Fig 2. Microlens cavity after microfabrication. a) top-down microscope image after electrode metal deposition and patterning. b) SEM image after microfabrication (no CYTOP film).

To conveniently evaluate the microlens performance we designed a 10 mm x 10 mm test chip with 44 individual microlenses. A completely self-contained microlens package was developed to enable optical characterization. To form the microlenses, the chip was lowered into a beaker containing the conducting liquid with a thin film of oil on the top. Through self-assembly, oil remained on the hydrophobic regions surrounded by the conducting liquid as the chip was lowered into the beaker. While submerged, a fused silica cap was lowered over the chip, encapsulating the microlenses. The chip was removed from the beaker, dried and sealed with epoxy. The sealed chip was wire bonded in a 40-pin DIP, which had a through-hole drilled in the socket region. Finally, the packaged microlens chip was inserted in a zero-insertion-force board mounted connector to allow for optical characterization.

3. Optical Characterization

To measure the focal length of the liquid microlenses as a function of applied voltage, we developed a novel approach based on beam magnification. The focal length is determined by the change in beam magnification the microlens imparts on a predetermined beam magnification optical system. The optical diagnostic system focuses a diagnostic beam through the test microlens with a ~16 µm spot size and outputs a beam with a size compatible with a beam profiling camera. In principle, if the microlens is aligned precisely in the optical diagnostic system then its

focal length could be determined directly from the measured beamsize. However, this is difficult in practice. Consequently, the microlens is also translated along the optical axis of the diagnostic system similar to a z-scan with beamsize measurements recorded at multiple positions. These additional measurements provide sufficient data to relax the alignment tolerance of the microlens, while improving the sensitivity of the focal length measurement. Initial characterization was done using a HeNe laser (λ = 633 nm). The microlens focal length is determined by fitting the measured beamsize as a function of translated distance to an exact analytical expression.

Figure 3 shows the focal length vs. voltage results from characterizing two different sized, fully-packaged, planar, single electrode, liquid microlenses. As the voltage is increased, the lens become more convex as water wets the electrodes and forces the oil into the center of the aperture, decreasing the effective focal length. Through active control of the focal length, the lenses can be ultimately steered to focus light on individual neurons at different depths in the brain.

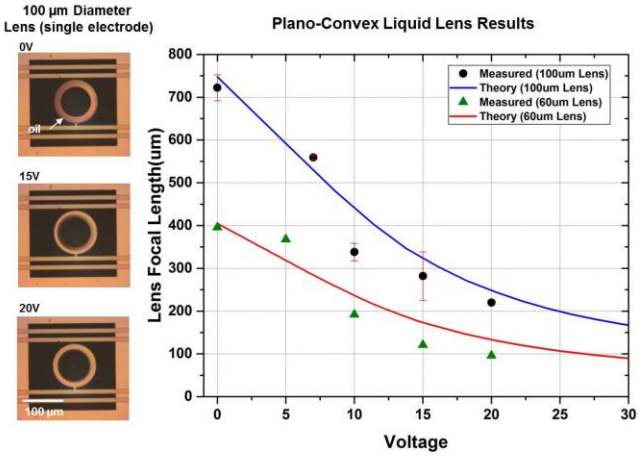

Fig. 3. Focal length vs. voltage for fully packaged, planar, single electrode liquid microlenses. The microlenses were sealed with DI for the conducting phase and DC-704 silicone oil. Top-down microscope images show the 100 µm diameter microlens shape at different applied voltages.

4. Conclusions

In summary, the results from the first phase of engineering micron-scale liquid lenses with active electronics to enable both focusing and steering have been presented. A novel design, that combines both focusing and steering into a single optical element has been developed with a liquid interface residing in a conical taper with interdigitated electrodes around the annulus. The tapered profile was generated using a gray-scaling lithography technique on a fused silica substrate. Optical characterization for planar microlens devices was completed, with a new measurement approach based on beam magnification that can be used for microlenses with micron size apertures.

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