Electrowetting lens for focus-tunable three-photon excitation microscopy

S. D. Gilinsky[1,*], D. N. Jung[2], G. L. Futia[3], M. Zohrabi[1], T. A. Welton[3], E. A. Gibson[3], D. Restrepo[4], V. M. Bright[2] and J. T. Gopinath[1,5]

1. Department Electrical, Computer, and Energy Engineering, University of Colorado Boulder, 80309, Boulder, USA
2. Department Mechanical Engineering, University of Colorado Boulder, 80309, Boulder, USA
3. Department Bioengineering, University of Colorado Anschutz Medical Campus, 80045, Aurora, USA
4. Department of Cell and Developmental Biology, University of Colorado Anschutz Medical Campus, 80045, Aurora, USA
5. Department of Physics, University of Colorado Boulder, 80309, Boulder, USA
Samuel.Gilinsky@colorado.edu

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***Abstract*—We demonstrate an electrowetting lens suitable for 3-photon microscopy using ionic liquids and 1-phenyl-1-cyclohexene. These devices are over 90% transmissive from 400-1600 nm and tunable from 133−48° with an applied voltage of 0−60 V. Using this device, we demonstrate 3-photon imaging at two focal planes.**

Keywords—Electrowetting, Tunable Lens, Multi-photon, Fabrication, Ionic Liquids

I. INTRODUCTION

Multi-photon fluorescence microscopy is a promising technique for high resolution imaging at deep focal depths in biological tissue [1]. Three photon excitation (3PE) microscopy has been shown capable of imaging at greater than 500 μm depths through an intact mouse skull [2]. The use of a longer wavelength (~1300 nm) reduces the impact of scattering and allows this technique to exceed the imaging depth limits present in one or two photon microscopy. Recently, a 3PE system was integrated into a head mounted microscope [3], allowing for neuronal imaging of a freely moving mouse and opening the door for groundbreaking research in memory formation and disease progression. Klioutchnikov *et al.* [3] used a mechanical z-scan to image multiple cortical layers at different depths inside the brain. While this technique showed large focal tuning with compact size and weight, mechanical scanning systems are prone to misalignment and eventual failure.

An attractive alternative is focus-tunable lenses based on the electrowetting on dielectric (EWOD) effect. These devices use an applied electric field to change the curvature of a liquid-liquid interface, resulting in a change in effective focal length. We typically utilize this effect by containing two immiscible liquids, deionized water (DI water) and 1-phenyl-1-cyclohexene (PCH), in a cylindrical glass cavity containing an electrode and dielectric layer attached to a base optical window with a ground electrode. As a voltage is applied between the ground and side electrodes on our cavity, the contact angle of the interface between the two liquids decreases and the curvature changes. If there is an index of refraction contrast between the two liquids, the device behaves as a focus-tunable lens. Tunable optical devices based on the EWOD effect have recently been demonstrated for multiphoton microscopy [4]. These devices are particularly attractive due to their compact and lightweight nature, low power consumption, and large tuning range. However, the use of DI water presents a significant challenge when attempting to integrate EWOD devices into 3PE microscopy systems as water has multiple absorption bands in the near-IR region. This is especially detrimental when dealing with the high peak power lasers typically used in 3PE microscopy systems.

We present an electrowetting lens design suitable for use in 3PE microscopy systems by utilizing room temperature ionic liquids (RTILs). These liquids have several key benefits. They have a negligible vapor pressure, high thermal stability, and, crucially, above 90% transmittance in the near-IR region. Electrowetting lenses using RTILs have previously been demonstrated [5], however these demonstrations used hazardous RTILs and dodecane as a nonpolar liquid, leading to a large density mismatch between the polar and nonpolar liquid and dangerous working conditions. We have chosen a non-toxic polar/nonpolar liquid combination of N-Propyl-N-methylpyrrolidinium bis(fluorosulfonyl)imide (N-Pr-Me-Pyrr $N(SO_2F)_2$) and PCH.

II. FABRICATION AND ASSEMBLY

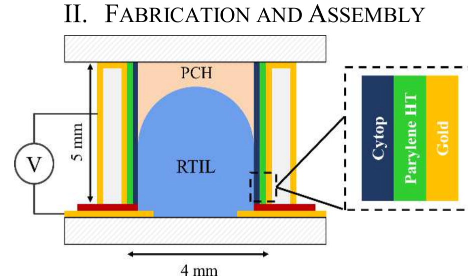

Fig 1. A two-dimensional schematic of a millimeter scale focus tunable lens based on the EWOD effect. The assembled device is filled with equal volumes of 1-phenyl-1-cyclohexene (PCH) and N-Propyl-N-methylpyrrolidinium bis(fluorosulfonyl)imide (N-Pr-Me-Pyrr $N(SO_2F)_2$.) A potential is applied between the walls of the cylindrical glass tube and the ground window which allows the curvature of the liquid-liquid meniscus to be controlled.

A two-dimensional schematic of a 4 mm inner diameter EWOD lens is shown in Fig. 1. Our design uses two separately fabricated components, a ground electrode window, and a cylindrical glass cavity with a uniformly deposited electrode used to contain the two liquids in our device as well as provide a potential difference between the polar liquid and the walls of our cavity.

The cylindrical glass tube and bottom electrode window are epoxy bonded with a UV curable epoxy (Norland UVS-91) and attached to a custom printed circuit board (PCB) allowing electrical connection to be made to our device with a pin connector. The final assembled device is filled using a micropipette with equal volumes (approximately 35 µL) of N-Pr-Me-Pyrr $N(SO_2F)_2$ and PCH.

III. Characterization and Imaging

Each device is actuated with a sinusoidal input voltage created by an analog output data acquisition card (DAQ, National Instruments PCIe-6738) which is amplified through a multi-channel amplifier (OKOTech).

A. Contact Angle Tunability

To characterize the contact angle tunability of our device, we position a CMOS camera parallel to the side of the cylindrical glass tube. Because the device is coated in a transparent electrode, ITO, we can take images of the liquid meniscus at various actuation voltages. Fig. 2a shows an image of an RTIL electrowetting lens when actuated to 0 V (top) and to 32 V (bottom). These images allow us to derive a relationship between contact angle and actuation voltage (shown in Fig. 2b). We observe that the contact angle of the liquid meniscus for our combination of N-Pr-Me-Pyrr $N(SO_2F)_2$ and PCH is tunable from $133°-48°$ with an actuation voltage of 0 V and 60 V, respectively.

B. Three-photon Excitation Imaging

We demonstrate the ability of our device to perform focus-tunable 3PE imaging by inserting into a benchtop 3-photon microscope. This system uses a 1030 nm center wavelength, 70 W average power regenerative amplifier (Spirit-1030-70, Spectra Physics) wavelength converted by a non-collinear optical parametric amplifier (NOPA-VIS-IR, Spectra Physics) to create a pulsed 0.8 to 1.1 W 1300 nm excitation source operated at 1 MHz repetition rate. The pulsewidth of this source is ~60 fs at the sample after passing through a prism compressor. The excitation source is directed through two galvo mirrors, scan lens, tube lens, and a 25x water-immersion objective (XLPLN25XWMP2, 25x/1.05 NA, Olympus) and focused onto the sample. Fluorescence and harmonic emission is collected back through the objective and reflected using a dichroic mirror from the excitation path, spectrally filtered, and detected by photomultiplier tubes (H10770PA-40, Hamamatsu). Data was acquired using Slidebook 2021 (Intelligent Imaging Innovations). We insert our electrowetting lens in the conjugate plane to the back aperture of the objective and actuate around 25 V, corresponding to 90° contact angle. To perform depth scanning, we actuate our device within a few volts around 25 V, taking an image at each focal plane. Images of a sciatic nerve excised from a ChAT-ChR2-YFP transgenic mouse are shown in Fig. 2c. The 3-photon fluorescence signal shows a subset of axons labelled with YFP. We recorded 3-photon images when our device was actuated to 26.5 V (left) and 25V (right) corresponding to a focal plane 10 μm deeper into the sample.

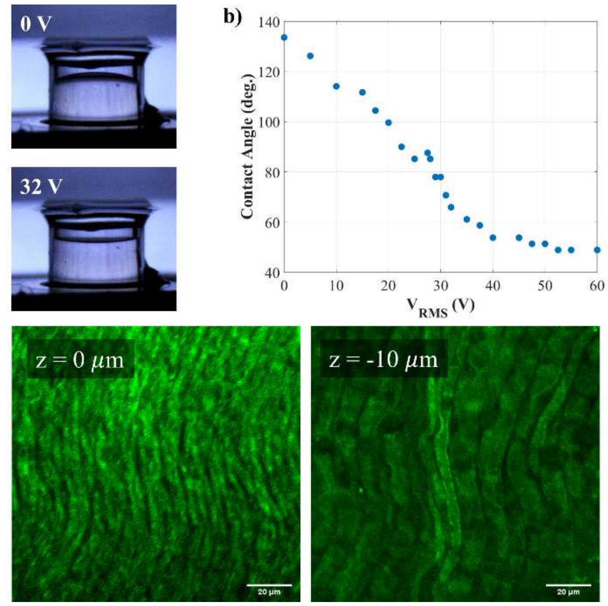

Fig. 2. (a) A 4 mm diameter electrowetting device filled with a liquid combination of N-Pr-Me-Pyrr N(SO2F)2 and PCH actuated at 0 V(top) and 32 V (bottom). (b) The change in contact angle of the liquid meniscus in our electrowetting device as we actuate from 0 to 60 V. Our device shows contact angle tunability from 133°–48°. (c) Three photon excitation images of ChAT-YFP in a mouse sciatic nerve taken through an electrowetting lens actuated at 26.5 V (left) and 25 V (right), corresponding to imaging 10 μm deeper into the sample.

IV. Conclusion

In summary, we present a novel EWOD lens design using a combination of the RTIL, N-Pr-Me-Pyrr $N(SO_2F)_2$, and PCH. We show that this liquid combination has greater than 90% transmission at 1300 nm and a refractive index contrast of $\Delta n = 0.1363$, justifying their use in 3PE microscopy systems. These devices can operate in both the diverging and converging lens regime, actuating from $130°$ to $48°$ with an actuation voltage of 0 V and 60 V, respectively. Finally, we demonstrate the capabilities of our device to perform focus-tunable 3PE microscopy by imaging in a mouse sciatic nerve at two different focal planes by actuating our device to 26.5 V and 25 V.

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