Electrowetting prism for scanning in two-photon microscopy

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Abstract: We have demonstrated an electrowetting prism as a lateral scanning element for a 2-photon excitation microscope. We show imaging of mouse hippocampal neurons, with a field of view of 130 × 130 μm^2.

OCIS codes: (110.1080) Active or adaptive optics; (180.2520) Fluorescence microscopy; (180.5810) Scanning microscopy.

1. Introduction

Laser scanners are an important component in high resolution microscopy. Often, standard microscopes utilize mirrors controlled by moving magnet galvanometers (galvo mirrors) for raster scanning [1]. However, with growing interest in miniature microscopes for *in vivo* neuronal imaging, there is room for developing alternative compact and low power technologies, that can be integrated into the microscope objective to provide lateral and axial laser scanning.

The electrowetting on dielectric (EWOD) principle enables control of the curvature of a liquid interface with an applied voltage, enabling tunable liquid lenses, prisms and customizable surfaces. EWOD adaptive optical technology offers a transmissive and low-cost alternative to other standard techniques. It has been demonstrated in miniature cameras [2,3] and displays [4], as depth scanning elements in confocal microscopy [5], and as adaptive prisms for non-mechanical beam steering [6–8]. In our work, we have integrated EWOD prism devices with repeatable and consistent scanning as slow axis scanner elements for 2-photon excitation microscopy [9]. We have also performed numerical simulations to evaluate the effect of propagating a Gaussian beam through the EWOD prism on the imaging quality.

2. Device fabrication and experimental set up

The EWOD prism devices are fabricated in cylindrical 4-mm inner diameter glass tubes that are 5 mm high. A novel 3D printing-assisted shadow masking technique is used to obtain two separated sidewall electrodes required for prism operation. The cylindrical glass tubes are coated with Indium Tin Oxide (ITO) as the sidewall electrodes, Parylene HT as the dielectric layer, and Teflon as the hydrophobic layer. The glass tube is epoxy bonded to an optical window with an annular patterned ground electrode (Figure 1(a)). The prism is filled with a polar liquid, 1% sodium dodecyl sulfate (SDS) aqueous solution, and a non-polar liquid, dodecane, and capped with an optical window. The assembled device and operation under applied voltage is shown in Figure 1(b).

Using Zemax and COMSOL, we have simulated Gaussian beam propagation through the EWOD prism and evaluated the effect of beam size on the imaging quality of the system. Based on the simulation results a beam size of 0.91 mm (full width half maximum) was chosen, resulting in a numerical aperture of 0.17 of the imaging system. The EWOD prism was integrated into a conventional 2PE microscope as a slow axis scanner, with the fast axis scan performed by a galvo mirror. The beam was conditioned to the desired size using a reverse Galilean telescope, and the scanned beam from EWOD prism is relayed to the galvo mirror using a 1:1 telescope. A commercially available EWOD lens is placed before the EWOD prism to compensate for its divergence.

3. Results

We imaged *in vitro* cultured mice hippocampus neurons, labeled with enhanced green fluorescent proteins (eGFP). A reference image was acquired using galvo scanners, and next, we replaced the slow axis galvo scanner, with an EWOD prism. Both images were acquired with 9 mW of average laser input power, at a resolution of 512 × 512 pixels with 10 μs of dwell time per pixel. The EWOD prism focal length also varies with actuation. This focal length shift with actuation is corrected by performing multiple scans with varying EWOD lens actuation voltage. This generates a stack of images with regions in focused resolved, and the whole image is constructed by taking a maximum intensity projection of the image stack.

From Figure 1(e), it is clear that the image collected using EWOD prism scan (Figure 1(d)) is in excellent agreement with reference image using the galvo scan (Figure 1(c)). In addition to the cell body, the high-resolution images were even able to capture the fluorescence expression from dendrites with width of the order of 5μm.

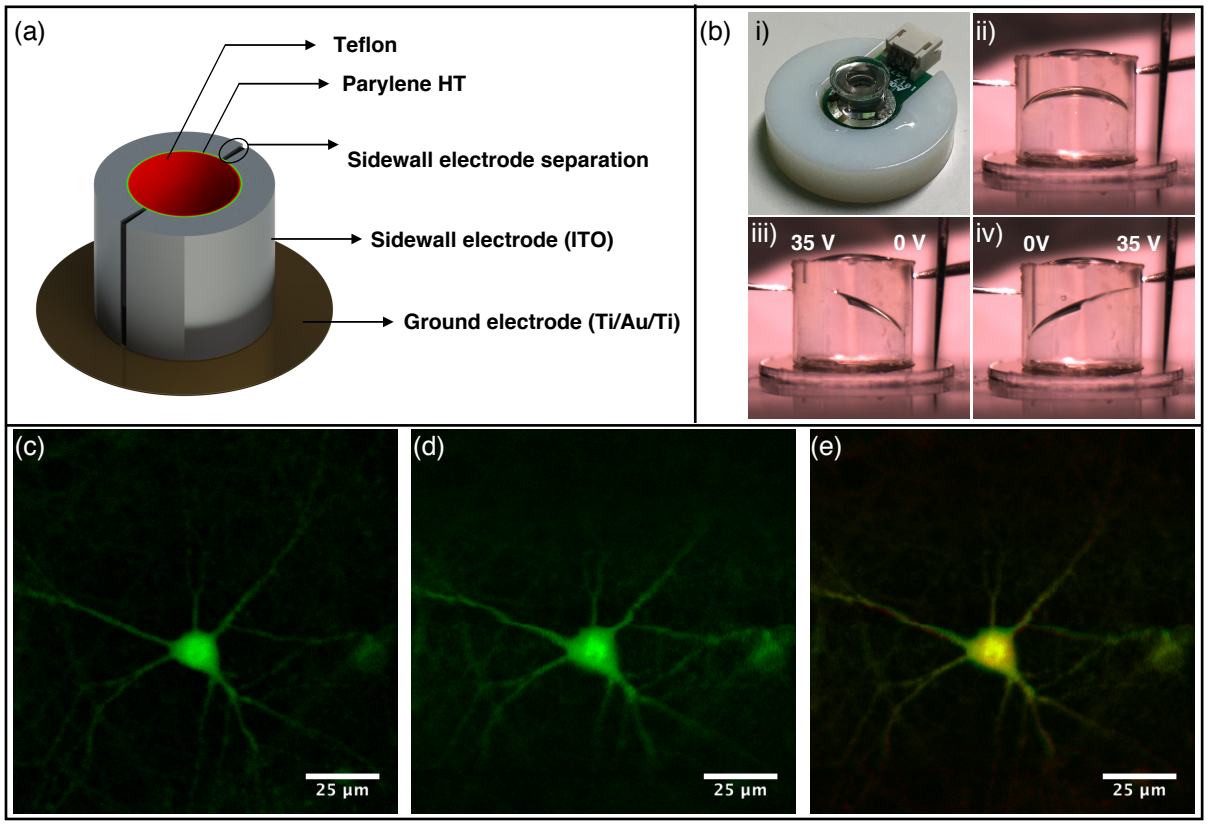

Figure 1: (a) Schematic of the device with components labeled. (b) Image of the assembled device and operation: (i) device bonded to a custom 1" mount, with liquids filled and capped with an optical window. (ii) Device operation at no applied voltage, (iii) 35 V applied to the left sidewall, and (iv) 35 V applied to the right sidewall. (c) Image of a neuron collected using galvo scanners. (d) Image of the same neuron collected using EWOD prism scanning. (e) Image (c) and (d) overlaid on top of each other, the red shade corresponds to the galvo scanned image, the green shade corresponds to the EWOD prism image, while yellow shade depicts the overlap region between the overlaid images. ITO: Indium Tin Oxide; Ti/Au/Ti: Titanium/Gold/Titanium.

4. Conclusion

We have demonstrated non-mechanical beam scanning in a 2PE microscope using a two electrode EWOD prism. 2PE microscope images of fixed mouse hippocampus neurons with a FOV of 130 × 130 μm^2 were acquired through EWOD prism scanning, with the EWOD prism acting as the slow axis scanner. In addition, the images were also able to capture the fluorescence expression from the dendrites connected to the cell body.

5. References

1. S. R. Schultz, C. S. Copeland, A. J. Foust, P. Quicke, and R. Schuck, "Advances in two-photon scanning and scanless microscopy technologies for functional neural circuit imaging," Proc. IEEE **105**, 139–157 (2017).
2. B. Berge and J. Peseux, "Variable focal lens controlled by an external voltage: An application of electrowetting," Eur. Phys. J. E **3**, 159–163 (2000).
3. S. Kuiper and B. H. W. Hendriks, "Variable-focus liquid lens for miniature cameras," Appl. Phys. Lett. **85**, 1128–1130 (2004).
4. R. A. Hayes and B. J. Feenstra, "Video-speed electronic paper based on electrowetting," Nature **425**, 383–385 (2003).
5. B. N. Ozbay, J. T. Losacco, R. Cormack, R. Weir, V. M. Bright, J. T. Gopinath, D. Restrepo, and E. A. Gibson, "Miniaturized fiber-coupled confocal fluorescence microscope with an electrowetting variable focus lens using no moving parts," Opt. Lett. **40**, 2553–2556 (2015).
6. D. Kopp, L. Lehmann, and H. Zappe, "Optofluidic laser scanner based on a rotating liquid prism," Appl. Opt. **55**, 2136–2142 (2016).
7. N. R. Smith, D. C. Abeysinghe, J. W. Haus, and J. Heikenfeld, "Agile wide-angle beam steering with electrowetting microprisms," Opt. Express **14**, 6557–6563 (2006).
8. S. Terrab, A. M. Watson, C. Roath, J. T. Gopinath, and V. M. Bright, "Adaptive electrowetting lens-prism element," Opt. Express **23**, 25838–25845 (2015).
9. O. D. Supekar, B. N. Ozbay, M. Zohrabi, P. D. Nystrom, G. L. Futia, D. Restrepo, E. A. Gibson, J. T. Gopinath, and V. M. Bright, "Two-photon laser scanning microscopy with electrowetting-based prism scanning," Biomed. Opt. Express **8**, 5412 (2017).