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Fast axial scanning for 2-photon microscopy using liquid lens technology

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Abstract

Scanning microscopy methods require movement of the focus in Z coordinates to produce an image of a 3-dimensional volume. In a typical imaging system, the optical setup is kept fixed and either the sample or the objective is translated with a mechanical stage driven by a stepper motor or a piezoelectric element. Mechanical Z scanning is precise, but its slow response and vulnerability to mechanical vibrations and stress make it disadvantageous to image dynamic, time-varying samples such as live cell structures. An alternative method less susceptible to these problems is to change the focal plane using conjugate optics. Deformable mirrors, acoustooptics, and electrically tunable lenses have been experimented with to achieve this goal and have attained very fast and precise Z-scanning without physically moving the sample. Here, we present the use of a liquid lens for fast axial scanning. Liquid lenses have a long functional life, high degree of phase shift, and low sensitivity to mechanical stress. They work on the principle of refraction at a liquid-liquid interface. At the boundary of a polar and an apolar liquid a spherical surface is formed whose curvature can be controlled by adjusting its relative wettability using electrowetting. We characterize the effects of the lens on attainable Z displacement, beam spectral characteristics, and pulse duration as compared with mechanical scanning.

Keywords

Remote focusing; Liquid lens; Active optics; Multi-photon microscopy

1. INTRODUCTION

Many biological studies require volumetric imaging of a feature or specific parts of a large sample. 2-photon microscopy [1] enables optical sectioning at relatively deep planes by exploiting longer wavelength excitation light. In the near infrared (NIR) and infrared (IR) regions, lower scattering contributes to a longer sample penetration depth. These characteristics make 2-photon microscopy popular in the biological research community. To use the microscopy modality and acquire 3D or 4D volumetric imaging, axial scanning is

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usually done in 3 ways: 1- mechanical scanning of the sample; 2- mechanical movement of the objective lens within its parfocal length; and 3- remote focusing.

Mechanical axial scanning using either stepper motors or piezoelectric actuators can precisely move and locate the sample at the desired position; however, they are usually slow methods. For faster axial scanning, another method employing piezoelectric objective drivers is used [2]. These methods enable very fast vertical sectioning. However, the fast mechanical movement can cause oscillation of the objective lens resulting in the addition of a wavy artifact to the acquired image. In addition, moving the objective out of its conjugate plane increases spherical aberration when imaging at the extremes of the objective range [3].

Several methods have been used to perform remote focusing using active or adaptive optics devices; these technique include variable focus lens by hydraulic pressure [4], electrically tunable lens [5, 6], acoustooptics [7], voice coil motor [8], deformable mirror [9, 10], and liquid lens [11, 12]. In this study, we focus on characterizing a liquid lens for 2-photon microscopy, which takes advantage of the electrowetting phenomenon.

Electrowetting is a phenomenon that changes the relative wettability of a liquid by applying electric potential. A liquid lens is constructed by combining two layers of conducting and non-conducting non-miscible liquids of the same density. The refractive index mismatch between the two, makes the boundary refractive, leading to a curved lensing surface. By changing the electrical potential between the two layers, the wettability of the liquids changes, which means that the surface tensions changes the curvature; hence a direct relation between the voltage and the optical power of the liquid lens is formed.

In this study we investigated the application of a liquid lens for remote focusing using 2-photon microscopy. We characterized the divergence and convergence for 3 pulse repetition rates with a constant average power but with different peak power, and simultaneously measured the temperature rise of the liquid lens. We further tested the ratio between Z scan range and the applied voltage. And finally we used the system for fast volumetric imaging of mouse brain slices.

2. METHODS

2.1 Optical setup

We placed the liquid lens at a conjugate back pupil plane (Figure 1). The 775 nm pulsed laser beam was restricted to 1 mm using an iris before the liquid lens (Varioptic Arctic-39N1), to make sure that we only use the active region of the lens, and also to reduce the amount of spherical aberration. We also use the collar on the objective lens to further reduce the spherical aberration. The beam was then magnified to enhance the numerical aperture (NA) of the system. We placed a beam profiler (Thorlabs M2MS & BC106N-VIS) between L_2 and the scanner to measure beam divergence and convergence.

The microscope is a home built setup modeled after our previous design [13, 14]. A 1550 nm, 370 femtosecond pulsed fiber laser (Calmar Cazadero) with wavelength of 1550 nm and repetition rate of 10 MHz was used. The beam was frequency doubled with a second

harmonic generation (SHG) crystal (Newlight Photonics) to produce a 775 nm beam for two-photon excitation of the sample. Power was modulated using a Pockels cell (Conoptics) and scanned over the sample by a resonant-galvanometer (fast axis – slow axis) scanner (Sutter instruments MDR-R). A 60x Olympus (LUMFLN60x) water immersion objective with NA of 1.1 was used for imaging. Z-scanning was performed using an X-Y-Z stage from the Sutter Instruments (MPC-200). Emitted SHG signal from the sample was collected using a 390/18 nm filter (Semrock). Photon multiplier tubes (PMTs) from Hamamatsu (H10770-40) were used for collection of the signal, and their signal amplified with a transimpedance amplifier (Edmund Optics 59-178). The system is capable of simultaneous 3 color imaging, but for these experiments, we only used one channel for acquisition (585/40 nm). A National Instruments DAQ card and FPGA module were used for control and synchronization of the system, and digitizing of the amplified SHG signal. The MATLAB-based Open-source software, Scanimage, [15] was employed to control the microscope.

2.2 Fluorescent bead volume

A 15% polyacrylamide gel containing multi-color 200 nm fluorescent beads was made by combining 90 μ L of 30% acrylamide with 90 μ L of 200 nm TetraSpec beads diluted 1:100 in ultrapure (18.2 M Ω /cm) water, 10 μ L Sodium Sulfite (10% w/v in ultrapure water) and 10 μ L Ammonium persulfate (10% w/v in ultrapure water). The solution was immediately poured on a #1.5 coverglass, and hardened at room temperature.

2.3 Brain section immunostaining and optical clearing

Staining and clearing of mouse brain slices were performed as described in [16]. The whole brain from a mouse (wild-type, C57BL/6J) was fixed in 4% PFA/PBS (–) overnight at 4 °C. Then, it was sectioned to 1 ~ 2mm thick slices using a brain matrix (Ted Pella). Brain sections were permeabilized, descaled and incubated in AbScale solutions (PBS(–), 0.33 M urea, 0.1–0.5% Triton X-100) for the staining of nucleus (NucBlue, Life Technologies, CA) and F-actin (ActinRed 555, Life Technologies, CA). Brain sections were cleared in Scale S0 solution (20% D(–)-sorbitol (w/v), 5% Glycerol (w/v), 1mM Methyl- β -cyclodextrin, 1mM γ -Cyclodextrin, 1% N-acetyl-L-hydroxyproline (w/v), 3% Dimethylsulfoxide (v/v), PBS, and pH adjusted to 7.2) for 6 days at 37°C. Each section was mounted with fresh Scale S0 solution for imaging.

3. RESULTS AND DISCUSSION

Characterization of divergence and convergence of the beam was our first step in characterization of the liquid lens for Z-scanning, to find a ratio between the voltage applied to the lens and the focal plane displacement. In Figure 2, we present the angle of divergence - and convergence where the angle is negative – versus the applied voltage. We made more measurements near zero, to find the intercept precisely. The results show a linear trend in a range between 30 V to 50 V. We repeated the test for 3 different repetition rates 30 MHz, 10 MHz, and 5 MHz. The average power of the 775 nm beam was kept constant at 100 mW incident at the liquid lens; which translates to 3.3 nJ, 10 nJ, and 20 nJ for 30 MHz, 10 MHz, and 5 MHz, respectively.

One concern during these experiments was that applying peak powers of 9 kW, 27 kW, and 54 kW on lens would increase the temperature and induce wavefront aberrations. We therefore placed a temperature sensor next to the liquid lens and measured the temperature during the experiment (Figure 3). By looking at the curve and the exponential fit on the acquired data, we observe that the temperature reaches a steady state after a few minutes of operation. Despite irradiating the lens with high peak power over a period of minutes, the temperature increase is small and does not go above the limit of 60° C. We further tested the spectrum of the beam before and after the liquid lens (Figure 4), which does not show significant beam broadening.

We made a volume of fluorescent beads in a gel for calibration of the scan range. We first mechanically scanned the volume with 0.25 μm step (figure 5a,b). And then scanned the volume again by changing the voltage of the lens (figure 5c,d). We looked at two planes in the first Z-stack, and compared them with their corresponding ones in the second stack. We found a ratio of 0.95 V/ μm for scanning, using our current setup.

We performed an experiment on a tissue cleared mouse brain slice, with cells stained with ActinRed (Figure 6). Figure 6(a) shows the whole volume of 64 μm x 64 μm x 35 μm was imaged at a rate of 1 μm per second (15 frames were acquired per second and averaged to increase the signal to noise ratio (SNR)). The volume of 512 x 512 x 35 pixels was acquired in 35 seconds with high SNR (bicubic interpolation was used to increase the number of Z slices to 140 for better presentation), compared to mechanical scanning which more than 1 minute. We then increased the rate of imaging, by not averaging frames and applying a steeper ramp signal to the liquid lens, and achieved imaging of a volume of 64 μm x 64 μm x 20 μm (512 x 512 x 20 pixels) in 1.33 seconds (Figure 6(b)). The image is noisier because of the lack of signal and due to reduced averaging, however this can be compensated for by better staining of the sample.

4. CONCLUSION

In conclusion, we characterized the liquid lens for fast 2-photon volumetric imaging, and applied it to imaging of tissue cleared brain slices. We measured divergence and convergence of the beam after the liquid lens and established a linear region usable for Z-scanning. We also measured temperature during operation of the liquid lens and noticed an exponential trend that goes to steady state, and does not surpass the limit of 60° C. We then imaged a volume of 200 nm fluorescent beads in polyacrylamide gel, and found a ratio of 0.95 $\mu\text{m}/\text{V}$. Finally, we tested volumetric imaging of brain slices in two slow and fast rates with high and low SNRs. In future work, we will characterize the wavefront error caused by the liquid lens, and apply it to functional intravital imaging.

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Figure 1.
Optical setup of the 2-photon microscope.

Figure 2.
Divergence vs. voltage at different repetition rates. (a) 30 MHz, (b) 10 MHz, (c) 5 MHz.

Figure 3.
Temperature of the liquid lens during the experiment.

Figure 4.
Spectrum before and after the liquid lens. At 10 MHz repetition rate.

Figure 5.
(a–b) mechanical Z-scanning, (c–d) voltage. Scale bars are 10 μm .

Figure 6. Volume image of mouse brain slice acquired using liquid lens. (a) 15 frames averaged for each slice to improve SNR. (b) The shown volume was imaged in 1.33 seconds.