# Non-invasive biopsy of doped-ions inside optical substrate by modified two-photon microscopy

**Yao-Chin Wang,[1,3] Hsiang-Ning Chen,[2,3] Wang-Yang Li,[2] and Ming-Che Chan[1,*]**

[1]*Institute of Photonic System, College of Photonics, National Chiao-Tung University, No.301, Gaofa 3rd Rd., Guiren Dist., Tainan City 71150, Taiwan*

[2]*Institute of Imaging and Biomedical Photonics, College of Photonics, National Chiao-Tung University, No.301, Gaofa 3rd Rd., Guiren Dist., Tainan City 71150, Taiwan*

[3]*Each author contributes equally to this work*

[*]*mcchan@nctu.edu.tw*

**Abstract:** Doped-ion based optical elements play key roles in optical signal processes, including amplification, absorption, wavelength-filtering, lighting, and polarizing plate. Non-invasively mapping the spatial distribution of the ion concentrations in these optical elements is highly desirable either during the fabrication process or to determine their optical qualities. In this work, we applied modified two-photon fluorescence (m-TPF) microscopy to trace the ion-distributions deep inside the optical elements. For demonstration purposes, polyvinyl alcohol (PVA) polymer films inside polarizing plates are taken as an example, where the spatial distributions of Iodine-dyed ions were measured by the m-TPF microscope in a fast and non-invasive way. The durability of the polarizer films can be distinguished from the axial distribution of the Iodine-dyed ions, without the need to perform a biopsy. This proposed method and demonstrated results show great potential for monitoring the spatial distributions of doped-ions in the optical elements quickly and non-destructively, which would be of great benefit in both scientific research and industrial applications.



**OCIS codes: (**110.2970) Image detection systems; (120.0120) Instrumentation, measurement, and metrology; (120.2040) Displays; (230.5440) Polarization-selective devices; (180.4315) Nonlinear microscopy.

## References and links

1. J. T. Verdeyen, *Laser Electronics* (Prentice Hall, 1995).
2. B. Zhou, D. Yang, H. Lin, and E. Y.-B. Pun, "Emissions of 1.20 and 1.38 μm from $Ho^{3+}$-doped lithium–barium–bismuth–lead oxide glass for optical amplifications," J. Non-Cryst. Solids **357**(11-13), 2468–2471 (2011).
3. P. C. D. Hobbs, *Building Electro-Optical Systems Making It All Work* (John Wiley & Sons, 2000).
4. J. A. Dobrowolski, G. E. Marsh, D. G. Charbonneau, J. Eng, and P. D. Josephy, "Colored filter glasses: an intercomparison of glasses made by different manufacturers," Appl. Opt. **16**(6), 1491–1512 (1977).
5. L. Wang, J. S. Swensen, E. Polikarpov, D. W. Matson, C. C. Bonham, W. Bennett, D. J. Gaspar, and A. B. Padmaperuma, "Highly efficient blue organic light-emitting devices with indium-free transparent anode on flexible substrates," Org. Electron. **11**(9), 1555–1560 (2010).
6. M. Seto, Y. Maeda, T. Matsuyama, H. Yamaoka, and H. Sakai, "Light Polarization in Iodine Doped Polyvinyl-Alcohol Films," Hyperfine Interact. **8**, 221–224 (1991).
7. E. H. Land, Light polarizer and process of manufacture, US Patent, 2328219, 1943.
8. V. Petrov, M. Cinta Pujol, X. Mateos, Ò. Silvestre, S. Rivier, M. Aguiló, R. M. Solé, J. H. Liu, U. Griebner, and F. Díaz, "Growth and properties of KLuW and novel ytterbium and thulium lasers based on this monoclinic crystalline host," Laser Photon. Rev. **1**(2), 179–212 (2007).
9. S. Ricaud, A. Jaffres, K. Wentsch, A. Suganuma, B. Viana, P. Loiseau, B. Weichelt, M. Abdou-Ahmed, A. Voss, T. Graf, D. Rytz, C. Hönninger, E. Mottay, P. Georges, and F. Druon, "Femtosecond Yb:$CaGdAlO_4$ thin-disk oscillator," Opt. Lett. **37**(19), 3984–3986 (2012).
10. M. S. Selima and A. Sawaby, "Optical studies on treated polyvinyl-iodine films and some applications," Polym-Plasti. Technol. **40**, 133–143 (2001).

#228958 - $15.00 USD Received 3 Dec 2014; revised 16 Jan 2015; accepted 16 Jan 2015; published 23 Jan 2015

11. E. J. Shin, W. S. Lyoo, and Y. H. Lee, "Polarizer effect and structure of Iodinated before and after casting polyvinyl alcohol film," J. Appl. Polym. Sci. **120**(1), 397–405 (2011).
12. W. Denk, J. H. Strickler, and W. W. Webb, "Two-photon laser scanning fluorescence microscopy," Science **248**(4951), 73–76 (1990).
13. J. M. Squirrell, D. L. Wokosin, J. G. White, and B. D. Bavister, "Long-term two-photon fluorescence imaging of mammalian embryos without compromising viability," Nat. Biotechnol. **17**(8), 763–767 (1999).
14. B. E. A. Saleh and M. C. Teich, *Fundamental of Photonics* (John Wiley & Sons, 1991)
15. P. N. Prasad, *Introduction to Biophotonics* (John Wiley & Sons, 2003).
16. S. P. Tai, M. C. Chan, T. H. Tsai, S. H. Guol, L. J. Chen, and C.-K. Sun, "Two-photon fluorescence microscope with a hollow-core photonic crystal fiber," Opt. Express **12**(25), 6122–6128 (2004).
17. H. W. Chen, T. Sosnowski, C.-H. Liu, L.-J. Chen, J. R. Birge, A. Galvanauskas, F. X. Kärtner, and G. Chang, "Chirally-coupled-core Yb-fiber laser delivering 80-fs pulses with diffraction-limited beam quality warranted by a high-dispersion mirror based compressor," Opt. Express **18**(24), 24699–24705 (2010).

## 1. Introduction

By doping various active-ions within transparent optical substrates, various optical functions, including optical amplification [1, 2], absorption [3], wavelength filtering [3, 4], solid-state lighting [5] and polarization distinction [6, 7], can thus be performed. First, the laser crystal, formed by active-ion-doping into a bulk crystal, is the key element for optical amplification. For example, the Ti:Sapphire laser crystal is a sapphire ($Al_2O_3$) crystal doped with Titanium ions. Second, for optical absorption and wavelength filtering, we use absorptive filters or colored glass filters, which are usually made from glass or plastics in which many absorptive active-ions have been added. These active ions transmit some wavelength components of light while attenuating others with extremely high absorption constants. For many filtering problems, these ion-doped wavelength filters are better choices than interference-type filters due to their much better wavelength extinction ratio [3]. Third, in white organic light-emitting diodes (OLEDs), the low-gap dopants are dispersed deep inside the emissive layer. Through electrical pumping, visible wavelength components are radiated in the emissive layer. Finally, for the fabrication of polarizing plates which are key elements in liquid crystal displays, Iodine-dyed polyvinyl alcohol (PVA) polymer films with a 1 to 20% Iodine concentration function as polarization-dependent light absorbers.

It is essential to monitor the spatial distribution and uniformity of dopants in laser crystals, color-glass filters, white OLEDs and polarizers. The doped condition of the laser crystal is strongly related to the laser performance, including the threshold pumping power, slope efficiency and output power [8, 9]. When low-gap dopants are not uniformly dispersed in the emissive layer of OLED devices, this can lead to spatial variation in the color of the white electroluminescence, affecting lighting applications. The spatial distribution of Iodine-dye in PVA polymer films may be helpful for extracting and mapping more properties of a polarizer [10, 11].

Traditional image analysis of doped-ion concentrations within an optical element requires a biopsy, including the removal, fixation, and staining of a piece sample from the transparent substrate. The sliced samples are then put under a microscope to measure and analyze the image to show the relative concentrations of dopants. Because the slicing procedures are invasive, destructive, and time-consuming, the relative ion concentrations cannot be monitored in real time during fabrication process with high accuracy. Thus, a fast and biopsy-free method for analyzing ion concentrations inside the above mentioned optical elements is highly desired. The method should provide highly penetrative, multi-dimensional imaging with a micrometer spatial resolution.

Recently, two-photon fluorescence (TPF) microscopy [12] has been widely utilized in biological, chemical, and clinical applications [13]. TPF processes usually involve fluorescent molecules with third-order nonlinearity, where two-excitation photons with equal energies are simultaneously absorbed by fluorescent molecules through the two-photon absorption (TPA) effect and one emission photon with a higher energy is generated. Molecular concentration imaging can thus be performed by measuring the intensities of higher-energy fluorescence

#228958 - $15.00 USD Received 3 Dec 2014; revised 16 Jan 2015; accepted 16 Jan 2015; published 23 Jan 2015

photons, with a natural depth discrimination capability and high spatial resolutions in the focal plane. However, TPF microscopy is not always suitable for monitoring the molecular concentration due to the non-radiative processes that occur in some doped-ions or molecules when they are excited by TPA effects.

In this study, a different approach, which we call modified two-photon fluorescence (m-TPF) microscopy, was utilized to trace the doped-ion concentrations deep inside transparent optical substrates. The TPA effect, proportional to the localized ion concentrations within a small volume near the focal plane, could be directly detected by measuring the intensity loss of the pumped photons (longer wavelength). In the experiments, Iodine-dyed PVA polymer films on polarizing plates were used as an example for demonstration purposes. By simply changing the relative position of the polarizer film and the focusing objective in the m-TPF microscope, the axial distribution of Iodine-dyed ions, utilized to distinguish the durability of a polarizer film, could be quickly and noninvasively measured in a non-invasive manner. Finally, 2D images of the relative ion concentrations, comparable to traditional microscopic images of sliced samples, were also obtained without any physical biopsy. These demonstrated results show that the m-TPF has great potential for monitoring the spatial distributions of doped-ions inside the optical substrate quickly and non-destructively, which will be of benefit in both scientific research and industrial applications.

## 2. Working principle and wavelength selection

Figure 1(a) illustrates the working principles and design of m-TPF microscopy by the input/output spectra. The input pump wavelength has a wavelength $\lambda$ and intensity of $I_0$. By the TPA process from the localized dopant deep inside the transparent substrate, output spectra of femtosecond pump and TPF are shown in the right hand of Fig. 1(a). In some conditions, instead of TPF, non-radiative processes dominate after the TPA of a pair of lower energy photons. Moreover, TPF signals with higher photon energies could be re-absorbed or may not penetrate the substrates. Due to these two reasons, in this experiment, localized ion concentrations were monitored through intensity loss of the pumping beam at the specified wavelength.

The layered structure of the polarizer film is shown in Fig. 1(b). From the surface of the polarizer film to the LCD cell, the structure includes a tri-acetyl cellulose (TAC) film with surface treatment, a uniaxial PVA layer, a wide viewing compensation layer (TAC), and a pressure sensitive adhesive (PSA) layer. These functional layers compose a complex multi-layered polarizer film. Except for the 25 μm thick uni-axially PVA layer, all other layers are highly transparent to visible and NIR light. In this experiment, two types of polarizers, durable and poorly-durable, were tested. In the durable polarizer, Iodine-dyed ions are spread uniformly throughout all the PVA polymer layers deep within the polarizing plates. The polarization extinction ratio can thus last for a long time. On the other hand, in the poorly-durable polarizer, the Iodine-dyed ions are clustered near both interfaces between the PVA layer and TAC layer. The clustered Iodine-dyed ions can easily diffuse to the TAC layer over time leading to degradation of the corresponding polarization distinguishing capabilities with the diffusion, which will influence the optical performance of liquid-crystal displays.

#228958 - $15.00 USD Received 3 Dec 2014; revised 16 Jan 2015; accepted 16 Jan 2015; published 23 Jan 2015

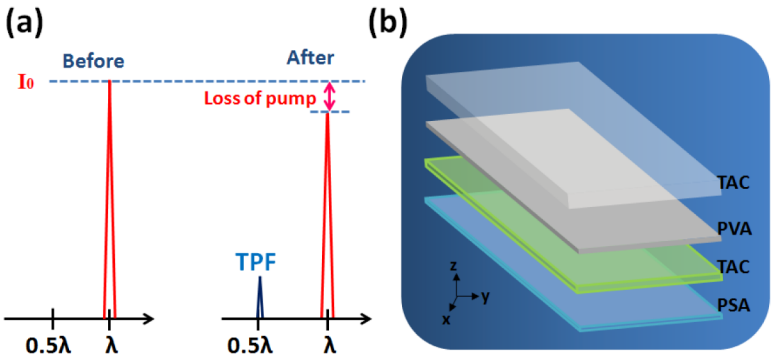


Fig. 1. (a) Principles of the ion-concentration mapping method. In this experiment, the loss in pumping was monitored to determine the doped-ion concentration. (b) The layered structure of a TN-type polarizer film. From the surface of the polarizer film to the LCD cell, the structure includes a tri-acetyl cellulose (TAC) film with surface treatment, a uniaxial polyvinyl alcohol (PVA) layer, a wide viewing compensation layer (TAC), and a pressure sensitive adhesive (PSA) layer.

The wavelength selection for m-TPF microscopy is very important in this experiment because it is related to the spatial resolution and depth sectioning capability. Excitation with shorter wavelengths contributes to a better spatial resolution in three dimensions [14]. However, if the excitation wavelength is too short, instead of the TPA effect, the linear absorption effect will occur, which will destroy the natural depth discrimination capability. Thus, before performing m-TPF microscopy, the transmission spectrum of the polarizing plate (sample) was first measured. From the measured spectrum, as shown in Fig. 2, the transmission efficiency is within the 30% to 40% range in the visible wavelength (400 nm to 780 nm) region. We attribute this to the fact that most photons in one polarization are linearly absorbed by the PVA layer of the polarizer and other photons with perpendicular polarization are transmitted. In the wavelength beyond 900 nm, the transmission efficiencies of the whole polarizer film increased to ~80%. Figure 2 indicates that in the region when the wavelength is shorter than 780 nm, the polarizing plate is highly absorptive, which corresponds to the linear absorption region. On the other hand, the polarizing plate is relatively transparent in regions where the wavelength is longer than 900 nm. When excited by a focused femtosecond laser beam with an output wavelength beyond 900 nm, the one-photon absorption effect was suppressed and the TPA effect only occurred near the focal point. Thus, the wavelength of the pump laser for the m-TPF microscopy should be longer than 900 nm.

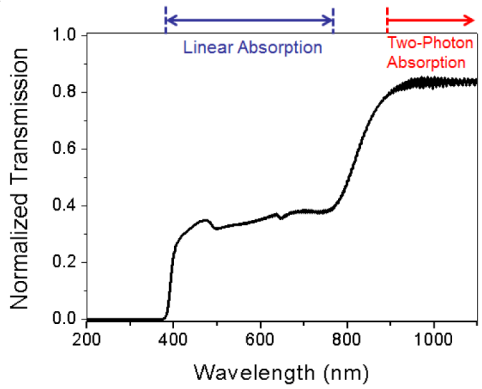


Fig. 2. Measured transmission spectrum of polarizing plate. The 400-800 nm regions are for one-photon absorption of Iodine-dyed layer in the PVA film and the wavelength regions beyond 900nm are suitable for two-photon absorption.

#228958 - $15.00 USD Received 3 Dec 2014; revised 16 Jan 2015; accepted 16 Jan 2015; published 23 Jan 2015

## 3. Experimental results

Figure 3 shows an outline of the experimental setup. As can be seen in Fig. 2, the pumping source for the m-TPF microscope was a compact Ytterbium laser with a 250 fs output pulse duration and 1.03 μm central wavelength. The polarization from the output laser beam was transformed into circular polarization by a quarter-wave plate. A pair of lenses ($L_1$ and $L_2$) were adopted for beam expansion so that the full aperture focusing objective ($OBJ_1$) was utilized to maximize the spatial resolution in the lateral (x and y) and axial (z) direction. The focusing objective ($OBJ_1$) had a 0.75 numerical aperture (NA). The residual pump (1030 nm) was then collected by another condenser ($OBJ_2$) with a 0.90 NA and focused into an unamplified detector (DET 36A, Thorlabs). The average power of the sample was decayed to 1 mW to avoid the photo-bleach and photo-damage of the iodine-dyed PVA polymer films. One long-pass color-glass filter (RG-850) was placed before the detector to block out possible unwanted visible wavelength noise from the background. By scanning the relative positions between the focusing objective ($OBJ_1$) and polarizing plate, the axial distribution of Iodine-ion-concentration in the PVA film was sequentially measured. From the basic Gaussian beam optics and experimental parameters, the calculated diffraction-limited spot size ($2\omega_0$), and the corresponding confocal depth, b, were 1.65 μm and 6.22 μm, respectively [14].

In the two-photon microscope, since the intensities of TPF or TPA are equal to the intensity square of the fundamental 1.03 μm pump Gaussian beam, the spatial resolutions in the x, y, and z directions are smaller than those in traditional microscopes [15]. The spatial resolutions in the x and y directions of the modified TPF microscope are 0.57 μm, which are equal to $\omega_0$ divided by $\sqrt{2}$. On the other hand, the spatial resolution in the z-direction, defined as the full-width-half-maximum of the TPF or TPA intensity, is equal to 0.643 b. In this experiment, the axial resolution is 4.13 μm.

Measuring the doped Iodine distributions in the depth (z) direction in real time is very helpful for understanding the polarizer properties because the axial distributions of Iodine-doped ion distributions are related to the durability of the polarizer. In a durable polarizer, the Iodine is nearly equally dispersed within the PVA layer. In contrast, in poorly-durable polarizers doped Iodine ions within the PVA layer aggregate in the interface between the PVA layer and TAC layer. The clustered Iodine-ions diffuse easily to the TAC layer over time and thus the polarization properties can easily vanish. This will lead to degradation of the contrast ratio and hue balance in liquid-crystal displays [11]. It is important to measure the depth-dependent Iodine traces during the fabrication process of the polarizer in real time, because the fabrication process could be terminated to save cost and time if unusual axial Iodine distributions are observed.

#228958 - $15.00 USD Received 3 Dec 2014; revised 16 Jan 2015; accepted 16 Jan 2015; published 23 Jan 2015

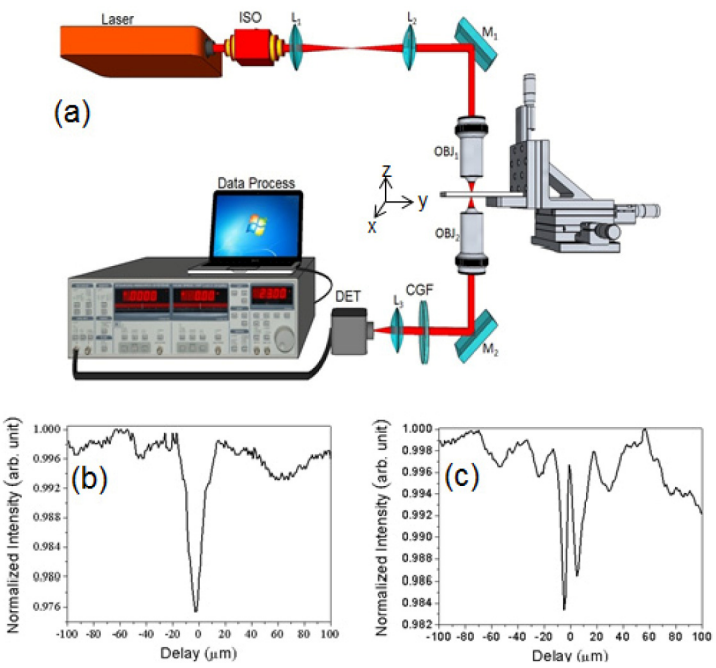


Fig. 3. (a) Schematics of the TPA microscope for monitoring the spatial distribution of doped ions. Here, Laser: Pump 1030 nm femtosecond laser; ISO: Isolator; $L_1$, $L_2$, and $L_3$: Lens; $OBJ_1$ and $OBJ_2$ are the focusing and collimating objectives; $M_1$ and $M_2$ are Mirrors; CGF: color glass filter; DET: detectors. Please note that the detector was linked to a lock-in amplifier to increase the signal to noise ratio; (b) the axial trace of a normal (well-durable) polarizer and (c) an abnormal (poorly-durable) polarizer. In (b) and (c), the step size was 1 μm.

Based on the points mentioned above, Figs. 3(b) and (c) show the depth (z) scanning traces of the durable and poorly-durable polarizers obtained with the m-TPF microscope. In the experiments, the z-step size was 1 μm. Based on the depth-dependent loss of the pumping intensity, the relative axial distribution of Iodine-dyed ions can be monitored and the durability of the polarizer can thus be predicted prior to packaging in a fast and noninvasively way. Figures 3(b) and 3(c) show the maximum decrease of intensity in the residual pumped photons, which was 2.5% and 1.7% in the durable and poorly-durable Iodine-dyed polarizers, respectively, that occurred due to the strong TPA effects in the Iodine-molecules. The measured width of Iodine-dyed layers, defined by when the TPA process starts, was 31 μm in both Figs. 3(b) and 3(c), which is in good agreement with a thickness of 25 μm for the Iodine-dyed layer and the calculated 4.1 μm axial resolution of the m-TPF microscope. The acquisition time for one depth-dependent pump intensity loss was about 1 minute, and was limited by the integration time of the lock-in amplifier (SR830, Stanford Research System) and the processing time of the controlling software (Labview 2010, National Instruments).

As can be seen from the measured z-traces in Figs. 3(b) and 3(c), the proposed method provides an efficient and effective way to monitor quality of polarizer films during the ion-doping process without the necessity of physical sampling, which is a destructive and very time-consuming process. Furthermore, the m-TPF microscope has the potential to be applied for noninvasively monitoring or predicting the optical quality of doped-ion-based optical elements, such as laser crystals and OLED-based lighting devices.

Although the z-dependent traces are enough to measure the durability of the polarizer, image-level comparisons are also shown in this experiment. Figures 4(a) and 4(b) show microscope images of the durable and poorly-durable polarizers where the PVA layers are

#228958 - $15.00 USD Received 3 Dec 2014; revised 16 Jan 2015; accepted 16 Jan 2015; published 23 Jan 2015

included. Figure 4(c) shows 2D virtual biopsy images of a polarizer in the x-z plane obtained with the m-TPF microscope. All the image sizes in Figs. 4(a) to (c) are 100 μm by 100 μm.

The invasive and time-consuming sample preparation procedures needed for measuring microscopic images such as those in Figs. 4(a) and (b), which include the removal, slicing, fixation, and observation of Iodine-dyed distribution in the PVA film under the microscope, cannot be easily executed without professional skills and special slicing machines. Figure 4(a) shows a microscopic image of the Iodine-dopants in a durable polarizer equally dispersed within the PVA layer. In contrast, in Fig. 4(b), in the poorly durable polarizer, the doped Iodine ions have aggregated in the interface between the PVA layer and TAC layer. Please note that, in addition to the time-consuming process, the contrasts in traditional microscopic images are relatively small, so that skilled operators are needed for the image determination for durable and poorly-durable films. In contrast, Fig. 4(c) shows the virtual biopsy images of a polarizer film in the X-Z direction taken by the m-TPF microscope. The images were directly recorded and linearly re-scaled with a computer. In Fig. 4(c), the distributions of Iodine-ions can be easily distinguished. In this demonstration case, most parts in the PVA layers are seen to be durable, since the Iodine-ions are evenly distributed throughout the whole PVA layer. In the image near the bottom of Fig. 4(c), a small portion of the PVA layer (enclosed in the dashed red circle) can be seen where the Iodine-ions are not equally dispersed within the PVA. There is a higher chance of forming white or black spots in the liquid crystal displays at these spots if the fabrication process is not terminated.

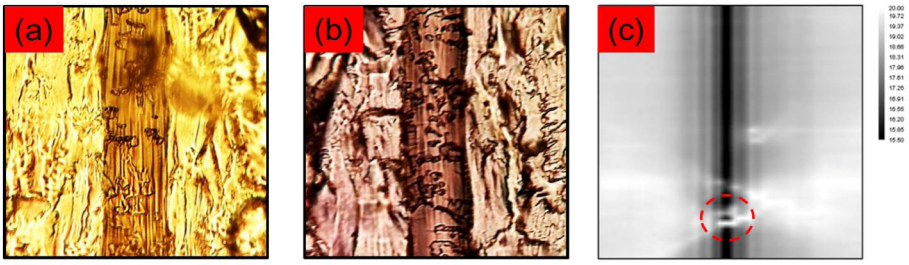


Fig. 4. Microscope images of sliced (a) durable and (b) poorly-durable polarizers. (c) The two-photon absorption virtual biopsy image of the test polarizer. Image sizes in these three figures are 100 μm by 100 μm. The PVA layers are vertically located in the center of the three figures with a 25 μm thickness.

## 4. Discussion and conclusion

The demonstrated results can be directly applied in the current liquid crystal industry and show great potential for producing high quality doped-ion-based optical elements. However, technically, there is still some room for improvement with this method, because of the 250 fs pump pulse duration and the 0.75 numerical apertures (NA) of the focusing objective. For image contrast, the maximum percentages of intensity decrease in the residual pumped photons can be raised by compressing the pulse duration of the light source. Under fixed and limited pulse energy to ensure sample viability, the TPA effects are inversely proportional to pulse duration [16] and can be improved several times by utilizing other powerful fiber lasers [17] with sub-100 fs pulse durations. The depth resolution, which is inversely proportional to the square of NA of the focusing objective, can be improved by replacing the microscope objective currently used with another air or water-immersion objective with a higher NA.

In conclusion, we have proposed and demonstrated a novel application of m-TPF microscopy for tracing the ion-distribution deep inside optical elements. The observation of the Iodine-ion concentration in PVA films deep within polarizing plates was used as a demonstrating example. The 1D and 2D spatial distributions of Iodine-dyed ions were measured by the m-TPF microscope in a fast and non-invasive way. The results show that the

#228958 - $15.00 USD Received 3 Dec 2014; revised 16 Jan 2015; accepted 16 Jan 2015; published 23 Jan 2015

durability of polarizer films can be quickly distinguished from the 1D axial distributions of Iodine-dyed ions observed through this non-biopsy method.

The demonstrated experimental results show great potential for monitoring the spatial distributions of doped-ions in optical elements in a non-time-consuming, fast, and non-destructive way, which will benefit both scientific researches by allowing more information about spatial distributions of doped ions to be known and industrial applications by finding problems during the fabrication process of these optical elements.

**Acknowledgment**

The authors also acknowledge the scientific discussions with Prof. Shie-Chang Jeng of National Chiao Tung University. This research was supported in part by the National Science Council of Taiwan (NSC 100-2221-E-009-092-MY3 and 103-2221-E-009-076) and the Department of Medical Research, Chi Mei Medical Center, Tainan 71004, Taiwan (CMCT-10305).

#228958 - $15.00 USD Received 3 Dec 2014; revised 16 Jan 2015; accepted 16 Jan 2015; published 23 Jan 2015