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Ultrafast holographic recording of snake infrared pit tissue using two-photon induced photopolymerization

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ABSTRACT

Non-cryogenic, high sensitivity infrared detection is one of the fundamental criteria for future sensor technology. To this end, we propose mimicking the natural infrared detection structures found in certain snake pit tissue. We hypothesize that the pit organ behaves like a photonic bandgap in that a regular arrangement of sub-micron micropits removes or traps visible radiation and enhances infrared radiation transmission. In order to simulate the two- and three-dimensional pit surface morphology, we used holographic two-photon induced polymerization (H-TPIP), a new microfabrication technique previously reported by this group. Using the ultrafast H-TPIP procedure, we can write large area biomimetic structures into an optical resin. Due to the quadratic dependence of the absorption probability on the incident radiation intensity, molecular excitation via the simultaneous absorption of two photons has been shown to lead to improved three-dimensional control of photochemical or photophysical processes. Using spatial variations in the incident intensity within a photopolymerizable resin, these structures can be readily fabricated. We report our progress on duplicating the surface morphology of snake infrared pit tissue using H-TPIP.

Keywords: holographic, two-photon, biomimetic, femtosecond laser

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1. INTRODUCTION

Biological infrared detection has been studied for over 60 years in the snake families Crotalidae and Boidae, pit vipers and pythons/boas, respectively[1]. Infrared or thermal detection is accomplished using specialized infrared pit organs that either line the jaw (boids) or are positioned on either side of the head below the eye (crotalines). Our research group has studied the surface morphology of these infrared pit organs using AFM and SEM[2]. This study indicated that there was a unique surface structure present in the infrared pits that was allowing the organs to scatter unwanted wavelengths of light and possibly enhance the transmission of desired infrared wavelengths of light.

We initiated a research project designed to duplicate the unique surface morphology of snake infrared pits in an effort to develop advanced optical coatings. We developed a process termed holographic two-photon induced photopolymerization (H-TPIP), the results of which were published last year[3]. This original H-TPIP produced structure was based on beam interference in a transmission grating geometry. Since this initial work, we have begun to determine how polymer film thickness varies in response to laser intensity. Additionally, we changed the holographic geometry from a transmission- to a reflection-based grating and experimented with post-cure techniques to preserve the reflection hologram.

We have successfully written a hologram of snake skin using a two-photon mediated photopolymerization process. This hologram is not of the pit organ, but the scales immediately surrounding the pit organ. The much larger structures of the scales surrounding the pit organ made it easier to validate the success of our technique. We report on our progress in recording biological structural information into a polymeric matrix.

2. EXPERIMENTAL

2.1. Film fabrication

For the formation of reflection-type holograms via H-TPIP, thin films were fabricated by pressing a 0.4 wt.% blend of the two-photon absorbing chromophore AF380 in NOA 72 between glass slides using commercially available Teflon spacers. To ensure solubility and dispersion of the chromophore within the monomer matrix, the chromophore was dissolved in a small amount of benzene prior to blending (~5% benzene by volume in final solution). The NOA 72 was passed through a 2 μm filter prior to mixing with the chromophore. The final solution was then re-filtered immediately prior to glass slide assembly. Film thickness varied with the type of Teflon spacer used. A spacer thickness range of 15 microns to 500 microns was used.

2.2. Optical configuration

The optical setup used to write the grating structures was a two beam holographic configuration. A Ti:Sapphire femtosecond laser system with regenerative amplification was used to generate a bandwidth limited ultrafast (typically 90 fs) pulse in the infrared (700-900 nm). High intensity, low dispersion optics were used throughout the experimental arrangement. The spot size was reduced from a square centimeter to several mm^2 using a pair of thin lenses in a telescope arrangement, and the beam was split into two pulses using an ultrafast beam splitter. It is important to note that the relative energies of the two pulses are <u>not</u> equal. The weaker of the two pulses is used for the patterning, while the more energetic pulse allows us to reach the threshold value for initiation of the process. This ratio is different per material system. Each of the two beams was propagated along optical delay lines, one of which was a variable translation stage with a resolution of 30 fs/step. For transmission type holograms, the two beams were incident on the sample plane at an angle of $\phi°$ to the normal, subtending an angle of $2\phi°$ between them. The grating spacing is then defined in the usual manner ($\Lambda=\lambda_o/(2\sin(2\phi))$). For reflection type holograms, the beams are nearly counter-propagating. As the coherence length of each pulse is approximately 30 μm, care must be taken to ensure accurate temporal and spatial overlap. To ensure temporal overlap, a KDP crystal is placed in the sample plane and the translation stage moved until the maximum of the second harmonic generation signal was determined. This process has the advantage of allowing us to also autocorrelate the pulse at the sample plane. An alternative method used is a thin film of a two-photon absorbing dye which upconverts the

fundamental pulses to a visible wavelength. Zero time overlap is then found by observing the bright flash of nonlinear fluorescence. By maximizing the temporal overlap, we also force the two beams into coherence with respect to each other. In addition, both the KDP crystal (TPA dye) and the sample holder are mounted on a rail to ensure that the interaction plane was identical by sliding either one or the other into the overlapping beams. The energy of each pulse is attenuated using thin neutral density filters, and is measured at the sample plane using a calibrated power meter. Both the energy and beam size is chosen to give an appropriate intensity distribution about the threshold intensity. Exposure times are determined by the kinetics of the reactions and can vary from as little as tens of seconds to as long as a few minutes. Although the grating spacing is determined from the subtended angle, the actual peak to valley dimension (Z) is determined by the intensity distribution about threshold of the generated pattern. To date, we have demonstrated a 50 nm peak to valley height[3], with large area patterns covering several mm^2.

2.3. Optical characterization

Once fabricated, holograms were examined using a CCD camera to measure diffraction efficiency and image formation in either transmission or reflection geometries at either 632.8 nm or using a fiber optic white light illuminator. In addition, transmission spectra of holographic gratings were obtained using an Ocean Optics fiber coupled spectrophotometer.

2.4. Film morphology and kinetics

Intensity dependent film thickness data were obtained using a Dektak IIA profilometer. The time dependent formation of the films was measured by propagating a low power helium-neon laser through the center of the film during the writing process. A silicon photodiode and A/D converter was used to collect real time transmission data during the formation of the photo-induced polymer film. Several sets of data were then collected as a function of pump intensity.

2.5. Chromophore excited state dynamics

The excited state dynamics of AF380 were measured using transient white light absorption spectroscopy. The experiment and results are presented elsewhere and so only relevant data are highlighted as needed[4]. In addition, a preliminary rate model of this chromophore is also presented in the same reference.

3. RESULTS AND DISCUSSION

The surface of a boid infrared pit organ is shown in Figure 1. This surface morphology is unique to the pit organ in several aspects. The distance between scale junctions is 3.5 µm and the scales are covered with micropits that also have unique dimensions. The scales at the junction points have greatly reduced, almost smooth, ridges compared to pronounced, finger-like projections elsewhere on the body. Experiments examining the optical properties of this tissue indicated that it scattered visible light much more readily than other tissues [5]. Our goal has been the duplication of this biological structure in a polymeric matrix. Two-photon technology is a natural complement to biological surfaces and matrices because of the greatly reduced intrinsic absorption of biological samples at these longer wavelengths. In this work, we attempted to accomplish this goal using holographic two-photon induced photopolymerization (H-TPIP).

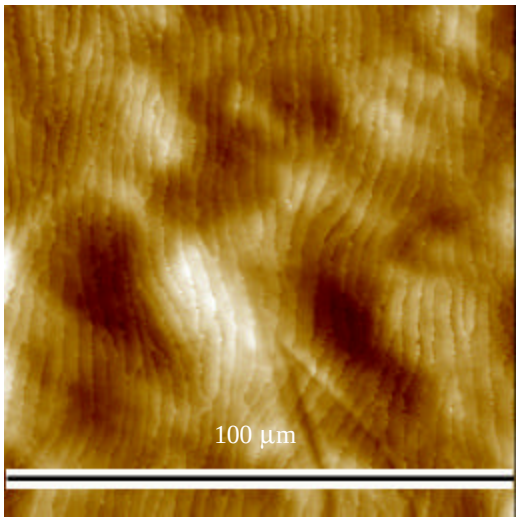

Figure 1 AFM image of boid infrared pit organ surface

Two-photon induced polymerization has been used to fabricate structures ranging in complexity from a spiral[6] to a photonic bandgap[7]. In these previous experiments, a fixed, confocal beam geometry was used to initiate two-photon polymerization while the sample stage was translated. By taking a holographic approach, entire areas can be written at once. This is analogous to parallel processing while the previous approaches were serial in nature.

Crotaline (rattlesnake) shed skin was mounted on a microscope slide. A Fresnel counter-propagating holographic setup was used to record information from the shed skin into polymer samples of varying thickness and the result is shown in Figure 2. With this particular reflection hologram setup, the image is written in planes that lie parallel to the grass substrate. The reflected image is the result of varying indexes of refraction created with these polymer planes. Regions of high tissue transmission corresponded to regions of high physical and holographic structure. The left-hand panel of Figure 2 depicts light regions that represent white-light scatter off of physical polymer structure. Darker regions in the polymeric structure correlated with a low degree of structure and corresponding low regions of transmission in the actual tissue sample.

Figure 2 Shed crotaline skin (right) and the corresponding holographic structure (left)

The holographic setup and the use of 100 fs pulse lengths made film thickness an extremely important experimental parameter. As the coherence length of a 100 fs pulse is ~ 30 µm, the proper choice of film thickness becomes critical with respect to the amount of three dimensional information stored. Too thin a

film will truncate three-dimensional information, while a film that is too thick results in a hologram at depth (or in bulk). Such a structure, while ultimately ideal for building larger three-dimensional objects, is difficult to convey visually without the aid of SEM, which was not practical at this time. We found for the case of these scale structures, that a 25 µm film gave the appropriate structure and level of detail necessary to convey in manuscript form (Figures 2 and 3).

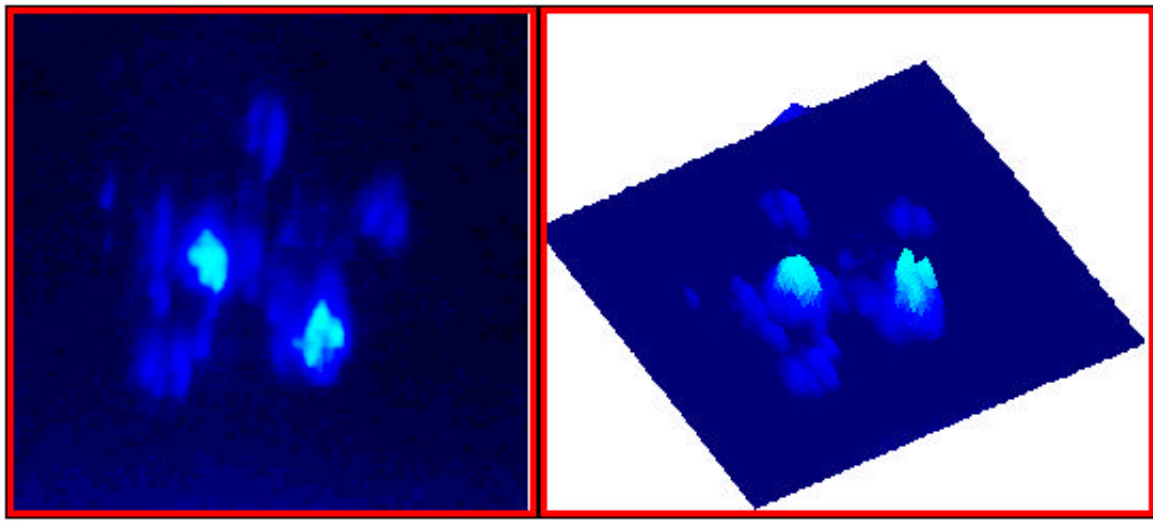

Figure 3: CCD camera images of snake scale holograms

In contrast to Figure 2, the images in Figure 3 show the holographic nature of the recorded image. A CCD camera was used to capture the reflected holographic image produced by illuminating the polymer film with a fiber optic white-light source. This holographic information overlapped with the physical polymeric structure shown in Figure 2. The different colors shown in Figure 3 reflect regions of varying diffraction efficiency. Confirmation that the CCD-recorded image was holographic is shown in Figure 4. At near-normal incidence the reflection hologram is clearly visible (Figure 4, right panel). At approximately 3º

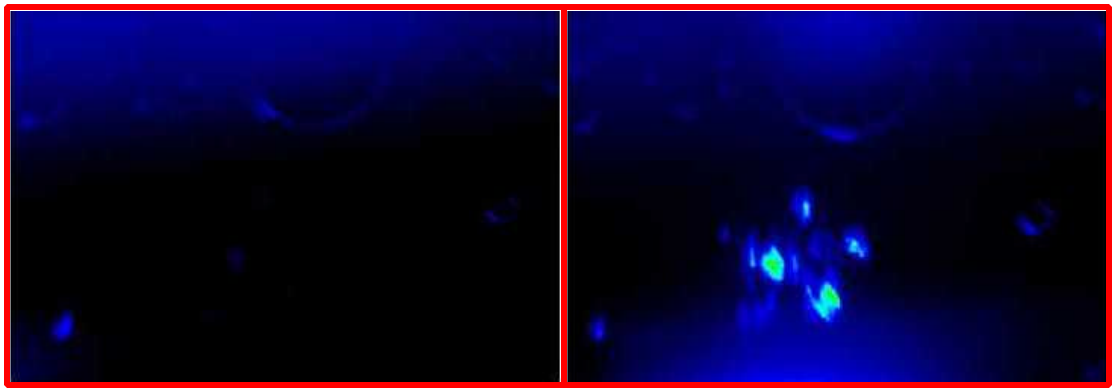

Figure 4 CCD camera images of the holographic regions at near-normal incidence (right) and 3º rotation

rotation, the image disappears. Additional measurements showed a reflection notch at the writing wavelength of 800 nm[8]. The polymer films were also examined using visible light microscopy (Figure 5). When examined using a long-pass filter (deep red transmission only), the polymer films showed evidence of substructure with features $\leq$ 2.5 µm. While further SEM analysis is required to correlate this polymer substructure with the tissue substructure, it is intriguing and encouraging that fine detail is being translated into the film.

There are numerous areas for future improvement. As mentioned earlier, polymer film thickness has not been optimized for this holographic approach. Rather than passing 800 nm light through the sample, we will be attempting to reflect light off of a silver-coated skin sample. This may eliminate some distortion caused by sample transmission. Additionally, these samples were prepared to prove the concept — future samples will be prepared in a clean room to eliminate dust-induced aberrations that were present in these samples. Finally, the components of this system can still be optimized. Research groups are producing chromophores with ever-increasing two-photon cross-sections and numerous photo-polymer blends that have not been explored using this technique.

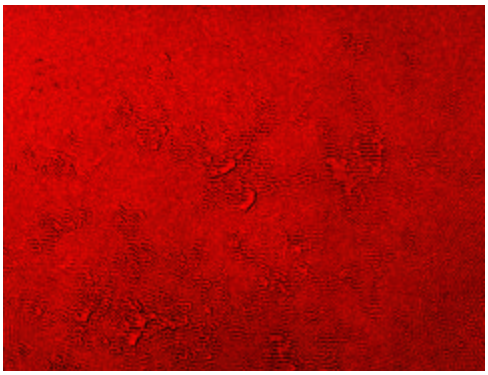

Figure 5 Optical microscope image at 400x magnification. Image is approximately 50 mm across.

4. CONCLUSION

We have successfully written a hologram of biological tissue into a photopolymer matrix using a two-photon process. The use of a chromophore with a high two-photon cross-section and the high peak intensity achieved with a fs laser made this accomplishment possible. We believe this technology will have commercial applications in the growing area of BioMEMS/NEMS. The ability to pattern polymers for micro- and nano-applications is critical to making MEMS technology more compatible with biological macromolecules. We have demonstrated that by using a holographic approach, it is possible to directly read biological information using near-IR light, and then record that information in a polymer matrix for later read-out. This application of two-photon technology is a natural complement to biological surfaces and matrices because of the greatly reduced intrinsic absorption of biological samples at these longer wavelengths.

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