Imaging three-dimensional microstructures fabricated by two-photon polymerization using CARS microscopy

T. Baldacchini*, R. Zadoyan

*Technology and Applications Center, Newport Corporation
1791 Deere Avenue, Irvine, CA 92606
tommaso.baldacchini@newport.com

ABSTRACT

Two-photon polymerization (TPP) is an enabling technology for the fabrication of three-dimensional microstructures with sub-micron resolution. Because of its unique set of advantages in respect of other microfabrication techniques such as photolithography, TPP has been employed in the last ten years for the manufacturing of devices in as diverse fields as microfluidics, microelectronics, photonics, and bioengineering. In this article, we exploit the imaging capabilities of CARS microscopy as a diagnostic tool for microstructures fabricated by TPP.

Keywords: photopolymerization, two-photon absorption, microfabrication, vibrational imaging.

1 INTRODUCTION

Among the arsenal of unconventional microfabrication techniques now available to scientists, two-photon polymerization (TPP) is unique for the fabrication of three-dimensional microstructures [1]. It is capable of producing geometries with no topological constrains and with resolution smaller than 100 nm. Because of its versatility, TPP has been employed to create devices previously impossible to manufacture with conventional microfabrication procedures such as photolithography. For example, by taking advantage of specific chemical moieties in the materials used in TPP, three-dimensional microstructures coated with conductors and semiconductors were fabricated delivering devices with distinctive and practical features for uses in microelectronics and nanophotonics [2, 3]. Furthermore, TPP is being used with promising results in biomedical applications by producing devices for drug delivery and tissue engineering [4].

TPP offers a unique combination of advantages: when fabricating three-dimensional microstructures, no topological constrains apply; sacrificial layers are not required in order to fabricate movable parts; by using laser intensities just above the polymerization intensity threshold, sub-diffraction-limited resolution can be attained. Although these features have been exploited successfully in diverse applications, there are still some fundamental questions that need to be answered. For example, how does the laser or sample scanning pattern used to fabricate a specific microstructure influence its mechanical properties? How can we optimize experimental conditions to minimize microfabrication time while maintaining structural integrity? At which dimensions do we have to consider the properties of the polymeric microstructures different from those of the bulk polymer?

Recently we have shown that coherent anti-Stokes Raman scattering (CARS) microscopy can be used to image microstructures fabricated by TPP and, more importantly, we have shown that some of the aforementioned questions can be answered by using this imaging technique [5, 6]. CARS microscopy is a nonlinear imaging technique capable of providing contrast with chemical selectivity [7]. Furthermore, because of the nonlinear optical nature of its light absorption, CARS microscopy allows for three-dimensional imaging.

Among a variety of approaches for performing CARS microscopy, we have chosen multiplex CARS (mCARS) microscopy [8]. The reason is that the experimental setup of mCARS shares the same laser source required for performing TPP. Thus, integrating TPP and mCARS microscopy in one experimental setup give us the opportunity to quickly investigate the process of TPP in situ.

2 MICROFABRICATION AND IMAGING

2.1 Two-Photon Polymerization

Two-photon polymerization is based on the nonlinear interaction of light with a photosensitive material (resin). Typically, near-infrared photons are used to induce two-photon absorption in molecules (photoinitiators) in the resin capable of starting a polymerization process. Since most photoinitiators posses small two-photon cross-sections, strong focusing lenses and ultrashort pulsed lasers are employed to increase the probability of this unlikely event. Under these conditions two-photon absorption and subsequent polymerization can be localized in a volume as small as a femtoliter. The ability of TPP to spatially confine matter transformation is the key step for three-dimensional microfabrication. Indeed, if the resin can be solidified by two-photon polymerization, complex geometries can be patterned with high precision by means of accurate positioning of the laser focal point. As long as the solubility properties of the solidified and unsolidified resin are
different, the non polymerized material can be washed away to leave the freestanding structures.

2.2 Coherent anti-Stokes Raman scattering microscopy

Coherent anti-Stokes Raman scattering microscopy provides three-dimensional imaging with contrast based on molecular vibration. In order to generate CARS, two laser beams are focused in the sample, a pump beam at frequency \( \omega_p \), and a Stokes beam at frequency \( \omega_s \). Vibrational sensitivity is obtained when the difference frequency \( \omega_d = 2\omega_p - \omega_s \) matches the frequency of a Raman active vibrational mode of the sample. The anti-Stokes signal at \( \omega_{as} = 2\omega_p - \omega_s \) is coherently driven by the two laser beams, resulting in coherent radiation. Traditionally, CARS microscopy has been performed by using two synchronized picosecond lasers as sources of pump and Stokes beams. In this way, imaging with high spectral resolution can be obtained. To acquire images in different spectral regions of the Raman spectrum of the investigated sample, the output wavelengths of the two lasers have to been adjusted accordingly. Alternatively, mCARS microscopy allows gathering the entire vibrational spectrum of the sample at once. In mCARS microscopy, the pump pulse has a narrow bandwidth which defines the spectral resolution of the technique. The Stokes pulse is spectrally broad, usually in the femtosecond regime. Application of the pump and Stokes beams excites simultaneously multiple Raman transitions within the bandwidth of the Stokes pulse. Thus, in a single shot the entire CARS spectrum of the sample exited states is generated.

3 EXPERIMENTAL SETUP

The experimental setup consists of three major parts (Figure 1a). A femtosecond oscillator (800 nm, 100 fs, 80 MHz), a unit for the generation of a pump and Stokes beams, and a microscope equipped with a laser scanning system. The microscope is completed with a detection scheme for collecting forward propagating signal by means of a photomultiplier tube (PMT). A detailed schematic for the unit which generates the pump and Stokes beams is shown in Figure 1b.

The broadband CARS unit is based a photonic-crystal fiber (PCF) which has a zero-dispersion wavelength at 800 nm. A portion of the laser output is used to generate supercontinuum in the photonic-crystal fiber. The near-infrared part of the supercontinuum (longer than 800 nm) is used as Stokes pulse. The remaining part of the laser output is spectrally narrowed to 3 nm by a bandpass filter and is used as pump beam. Pump and Stokes beams are then overlapped both in space and in time with the aid of a razor edge long pass filter (RELP) and a delay line. Each arm of the setup has a variable attenuator which allows independent control of intensity and polarization. The collinearly propagating beams are directed into the microscope where a pair of galvonomic mirrors is used to scan the sample. One focusing objective is used for both imaging and fabrication (40x, 0.75 NA).

TPP is performed by removing the 3 nm bandpass filter from the path of the pump beam and by blocking with a shutter the Stokes beam. Three-dimensional structures are patterned with the aid of the galvos and a piezo-focusing stage. The sample consists of an acrylic-based photoresist that undergo radical polymerization when irradiated by femtosecond laser source centered at 800 nm[9]. After fabrication, the microstructures are revealed by washing away the unsolidified portion of the sample with an organic solvent.

![Figure 1](image)

**Figure 1** – (a) Block diagram of the experimental setup employed for both TPP and mCARS microscopy, and (b) schematic drawing of the broadband mCARS unit. FI: Faraday isolator; ½ WP: half-wave plate; BS: beam splitter; PCF: photonic crystal fiber; LP: long-pass filter; RELP: razor edge long pass filter.

4 RESULTS AND DISCUSSIONS

Scanning electron microscopy (SEM) images of representative three-dimensional microstructures fabricated by TPP are shown in Figure 2. The truncated cone with square cross-section in Figure 2(a) was created by stacking in the z direction square patterns with smaller and smaller diameters. A separation between the different stacks of 3 \( \mu \)m was selected and a total height of 60 \( \mu \)m was reached. The laser average power (as measured before the shutter), stages velocity, and excitation wavelength used for the fabrication process were 10 mW, 10 \( \mu \)m/s, and 775nm, respectively. A more complex microstructure is depicted in Figure 2(b). Two towers were fabricated one inside the other; a larger one with a hexagonal base and a smaller one with a square base. At the top of each microstructure, two freestanding beams connect adjacent sides. Since the outside tower is 40 \( \mu \)m tall while the insider tower is 20 \( \mu \)m tall, there is no overlap among the two sets of beams. The
experimental conditions used for the fabrication of the microstructure in Figure 2(a) were repeated for the fabrication of the microstructure in Figure 2(b).

Figure 2 – SEM images of microstructures fabricated by TPP. The samples were tilted 45° in order to reveal the three-dimensional nature of the microstructures. A top view of the microstructure in (b) is shown in the inset. The polymeric beams that run across the towers in Figure 2(b) are 4 μm wide.

In order to assess the capabilities of the broadband CARS unit, a test sample was fabricated by TPP. First a set of four walls 35 μm long and 20 μm tall were fabricated. Then, a cross-like structure 20 μm tall was patterned within the walls. Finally, the cube was closed by fabricating a thin roof on top of it. A schematic diagram of the microstructure is depicted in Figure 3(a); it consists of a cube with four chambers filled with unpolymerized photoresist. A scanning electron microscope of the microstructure is shown in Figure 3(b).

Since the photoresist used in this experiment is rich in carbon-hydrogen (C-H) bonds, we placed in front of the PMT used in collecting forward CARS signal a 10 nm bandpass filter centered at 650 nm. This spectral range corresponds to the CARS signal originating from the C-H stretching mode (~ 3000 cm⁻¹). Thus, when imaging the structure shown in Figure 3(b) with broadband CARS microscopy, the signal intensity is correlated with the local density of C-H bonds.

Figure 3(c) is a xy cross-section CARS image of the cube recorded at a height of 18 μm from the substrate. Stronger signal is observed from the walls and cross-like structure than from the inside of the pockets that contain unpolymerized material. Furthermore, because of the proximity of the scanned area with the roof of the microstructure, part of the signal in image shown in Figure 3(c) arises from the roof itself. Upon TPP, the photoresist degree of cross-linking increases enormously. Thus, the density of the polymerized photoresist is higher than the density of the unpolymerized photoresist. Broadband CARS microscopy has revealed this material properties change with high spatial resolution, rapidly, and by using the same experimental setup employed for TPP.

5 CONCLUSION

We have demonstrated the use of broadband CARS imaging for the microscopic characterization of three-dimensional microstructures fabricated by TPP. Both microfabrication and imaging were performed on the same
experimental setup allowing for fast in situ monitoring of TPP. This result demonstrates the diagnostic potential of CARS microscopy in analyzing written patterns in the native photoresist in real time.

REFERENCES