Two-photon Polymerization Process for Optically Driven Micromachines

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ABSTRACT

Exposure of optically curing resin with highly focussed femtosecond laser pulses provides excellent means to produce high resolution micron sized structures. We use the process to fabricate micromechanical components for lab-on-a-chip applications. We present here our experimental realization of the microscope system used for photopolymerization and detail the advantage of our fabrication process. We characterize our structures using scanning electron microscopy, and compare the results with available data. We demonstrate the technique by manufacturing a movable joint and a free floating cross which is three dimensionally trapped. Future applications of this technique will focus on developing optically driven motors and an all optical measurement of applied torques.

Keywords: two-photon, polymerization, micromachine, micromanipulation, resin, femtosecond, optical tweezers

1. INTRODUCTION

Commercial microscopic mechanical elements are mainly produced with lithographic processes, in which a multitude of layers are produced, cured and etched (Micro Electro Mechanical Systems, MEMS). For advances in the development of a lab-on-a-chip for biological under the microscope applications, transparent micromachines are required, which can be operated on a simple microscope slide. Laser light as a driving force for these machines is preferential, since it can easily be coupled into the microscope. To prevent heating and allow optical trapping, machine components need to be transparent.

In this paper, we use optically curable resin and two photon polymerization to produce microscopic 3D machine elements. We address the experimental requirements and procedures to achieve this, and detail the advantages. We investigate the properties and resolution of this femtosecond exposure system with electron microscopy. We use in situ development of the produced structures to allow fabrication and optical trapping of a single structure. The first structures to be produced here are a micro joint and a micro cross.

Since the start of the production of microscopic elements with two-photon polymerization,1 many innovations have been made. Meeting gearwheels on axles were produced and driven with a microfabricated rotor.2 Micro-needles and micro-tweezers with sub micron tips were produced with high resolution and an impressive accuracy.3 These tools were hinged on fixed posts and could be moved with optical tweezers. More recently, microscopic cogs with intrinsic form birefringence were produced using electron beam lithography and spun with precise position control in optical tweezers.4 These studies demonstrate the impressive advances which have been made over recent years and suggest potential applications which yet have to be explored.

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2. EXPERIMENTAL METHODS

2.1. Two-photon Polymerization

We use the UV curing resin NOA63 (Norland Products Inc., Cranbury, NJ, USA) to produce the microstructures. Photopolymerization of the resin occurs when it is exposed to light in the UV wavelength range (here $\lambda < 400 \text{ nm}$). Light curing resins usually contain an initiator and monomers/oligomers, e.g. urethane acrylate. The initiator absorbs an UV photon and becomes a radical. The radical can break carbon double bonds in the monomers, which generates more radicals. Monomer radicals bind to other monomers and a polymerization chain reaction takes place. The reaction stops if chained radicals meet.

Curing is usually performed with UV light sources, the power required for a full cure of NOA63 resin is 4.5 J/cm$^2$ (see NOA63 application notes). We use infrared femtosecond laser pulses for curing the resin (wavelength 780 nm). To produce radicals, two photons have to be absorbed simultaneously in the so called two-photon absorption (TPA) process. This process is very unlikely compared with normal absorption. Thus, TPA can only be observed at places of high photon intensity, like in the focal spot of a focussed laser beam. This has the advantage that other areas in the resin, even though exposed to the propagating high intensity infrared laser beam, do not cure, and high resolution structures can be polymerized. TPA is a nonlinear process and thus scales with the square of the intensity.

2.2. Experimental Setup

We perform two-photon polymerization in a custom build inverted microscope. This enables easy access to all optical elements and allows great versatility. The system is simultaneously used for optical trapping of fabricated structures. Figure 1 shows a detailed sketch of the system. For polymerization, a Ti:Sapphire laser (Tsunami, Spectra Physics Inc.) is used. It is pumped by a 532 nm 10 W solid state laser (Millennia, Spectra Physics Inc.)
Figure 2. Scheme of the photopolymerization procedure employed for microfabrication. The optically curable resin is sandwiched between two cover slips. The femtosecond laser beam is introduced from below by a high NA objective lens. The sample is raster scanned and the resin exposed according to a 3d matrix which contains the data for the production of the structure. Growth of the structure starts at the upper cover slip, meaning the structure grows upside down, which has the advantage that the laser does not pass through already exposed resin, which could deflect the beam. After exposure, the unexposed resin is washed out with acetone.

Inc., Mountain View, CA). The laser beam is expanded to match the back aperture of the objective lens. It is then attenuated to the optimum power to achieve good polymerization and prevent excessive heating. It passes through a computer controlled shutter and is reflected by a dichroic mirror into the objective. We use an Olympus 100× oil immersion lens with a numerical aperture of 1.3 to achieve the smallest possible spot size and the highest spatial resolution for photopolymerization.

Between objective and dichroic mirror, another dichroic mirror is used to couple the trapping beam into the system. This mirror transmits 800 nm and visible light, but reflects 1070 nm. The trapping laser is a 5 W, 1070 nm fiber laser (model YLD-5-LP, IPG Photonics, Oxford, MA), its output power is controlled with a half wave plate (\(\lambda/2\)) and linear polarizer. A quarter wave plate is used to obtain circular polarization (\(\lambda/4\)). The mirror M1 is gimbal mounted and imaged onto the back aperture of the objective to allow precise position control of the optical trap.

Samples are mounted on a computer controlled piezo actuated stage and imaged by bright field microscopy using the same objective lens.

2.3. Fabrication Procedure

Two dimensional structures attached to the coverslip can easily be grown by raster scanning the sample with the resin over an area using the piezo actuated and computer controlled microscope stage. The shutter opens to expose the resin at the correct time to obtain the desired structure. Three dimensional structures are obtained by offsetting the sample chamber in Z-direction before the next X–Y scan (Fig. 2). We have developed a control program which reads in a bitmap file (corresponding to the scanned area) and exposes when the pixel is set to black. The bitmap resolution of 100x100 pixels corresponds to 18 \(\mu\)m travel, giving the individual pixel a size of 180 nm. As each layer can be addressed with an individual bitmap, complex 3D structures can be fabricated.

We fabricate 3D structures in a sample chamber which is enclosed by either two coverslips, or one coverslip and a microfluidic device made from a PDMS cast of a SU8 master waver. Using two coverslips has the advantage that the structure can be grown upside down on the upper coverslip. The first layer is immediately attached to the coverslip which prevents the deformation of thin structures due to viscous flow in the resin. Furthermore, the laser beam does not have to pass through already exposed areas, which have a different refractive index, and is therefore not deflected.

Typical parameters for polymerization are an average power of 20 mW in the specimen plane, a wavelength of 780 nm and a pulse length of 80 fs (at 80 MHz repetition rate). Depending on the size of the structure, scanning a plane takes between 20 and 40 seconds. More complex 3D structures can take 30 minutes to fabricate.
Figure 3. Scanning electron microscopy and light microscopy images of structures produced by fs laser photopolymerization. (a): A raster of lines with different width is used to evaluate the width of the region in the focus in which polymerization occurs. (c): Information about the height of the exposed region is obtained by using light microscopy to observe a tipped over structure (here a 3d cross). The asymmetric arms of the cross already show a bigger exposed region along the Z-axis compared to X and Y. (d) and (d): Comparison between the production of a structure (an Australian national symbol) with a 40× air objective (NA=0.65, (b)) and a 100× oil immersion objective (NA=1.3, (d)). The higher resolution due to the tighter focal spot of the high NA objective can be clearly seen. Individual pixels of the raster scan with a grid spacing of 180 nm can be observed.

The structure is developed by washing the unexposed resin off with acetone. When the structure is attached to the coverslip, this can be readily done, and the acetone is subsequently dried out and replaced with the chosen fluid. If the structure is free floating, we use the microfluidic PDMS sample chamber, and slowly infuse the acetone to prevent strong currents in the sample. Simultaneously, the produced structure is trapped with the optical tweezers system. The acetone dissolves the resin and fluid viscosity and refractive index around the structure change slowly. Lower refractive index leads to higher trapping force on the structure, but hydrodynamic currents due to fast diffusion of the resin can occasionally wash the structure out of the trap so that it is lost.

2.4. Characterization

We use electron microscopy for the characterization of produced structures. Test structures are grown on coverslips, developed and stored. The coverslips are then mounted on an adhesive carbon pad, and a 10 nm platinum layer is sputtered onto the sample. Structures are investigated with a field emission scanning electron microscope (JEOL JSM-6400F). The resolution of better than 2 nm allows the precise characterization of the structures.
3. EXPERIMENTAL RESULTS

System Performance

The system is capable of producing high resolution complex 3D structures. Growth times for a complex $8 \times 8 \times 5 \, \mu m$ structure is on the order of 25 minutes. Since the laser power is strongly attenuated at the moment, higher scan speeds should be possible. Structures generally adhere strongly to the glass substrate, and do stay attached even during high flow rate washing. We have also produced free floating 3D structures. *In situ* development of the structure within a microfluidic device allows production and trapping of a single structure. The process is being optimized at the moment.

Resolution of Polymerization Process

The resolution was first checked by comparing structures produced with a $40 \times$ air objective and a $100 \times$ oil immersion objective (Fig. 3 (b) and (d)). This shows the importance of using a high numerical aperture (NA) objective (0.65 vs. 1.3) to obtain a tight beam waist in the focal region. A small diffraction limited spot is obtained, if the polymerization laser beam overfills the back aperture of the objective.

We performed a quantitative study of the resolution of the photopolymerization process with SEM. Lines with a width of 1 to 5 pixels and a height of three layers were polymerized (Fig. 3 (a)). The line with a width of only one pixel was detached during the development process, leading to the assumption that it may not be feasible to produce such delicate structures as they do not withstand the hydrodynamic forces in the development process. The width of the lines was measured from the electron microscopy image (Fig. 4). The linear fit to the data allows an extrapolation to the single pixel width. It was found to be $290\pm20 \, nm$. A similar evaluation of the axial resolution was not possible at the time since structures could only be observed from above under the SEM.

To obtain the axial resolution, we investigated a 3D cross under the light microscope (Fig. 3 (d)). The uncorrected length of an arm of the cross was 9 pixel ($1.44 \, \mu m$), whereas the uncorrected height of the arm was 7 pixel ($1.1 \, \mu m$). Uncorrected means here without taking the size of the exposed spot into account. One can immediately see that length to height ratio is not as expected, which is due to a non spherical but strongly spheroidal shape of a single exposed pixel. Calibration and evaluation of a number of images revealed an axial spot diameter of $1.2\pm0.2 \, \mu m$. The ratio of axial to horizontal spot size is therefore 4:1.
Figure 5. The gray ellipsoids depict the area which is polymerized when the sample is scanned over the raster of white dots, where the resin is exposed to the fs laser radiation. The strong elongation of the focal region in which polymerization occurs causes the width to height ratio of a produced structure to change. This needs to be taken into consideration when designing micromechanical structures. Structures with high axial resolution can still be achieved by stacking layers as shown here.

Figure 6. Moving joint produced with photopolymerization. The width of the structure is 16 µm.

Figure 5 illustrates which areas are actually exposed during a raster scan. We have modified our exposure software so that the shutter does not close between exposure of subsequent pixels, so that we obtain smooth lines instead of the here depicted hill and valley structure.

Fabrication of Moving Structures

We have produced a micromechanical joint (Fig. 6). The joint was polymerized in the microfluidic sample chamber and \textit{in situ} developed. After development, the joint could be moved with optical tweezers.

In a similar procedure, an asymmetric cross was manufactured. The cross could be rotated and finally aligned in with its long axis along the beam axis.

4. DISCUSSION

Two-photon photopolymerization is a very advantageous process for the fabrication of microstructures. The main advantages can clearly be seen from the analysis of the structures we produced. These are as follows. Polymerization occurs only in a well defined volume in the focal spot. The dependence of two-photon processes
Figure 7. Intensity distribution along horizontal ((a) and (b)) and along axial ((c) and (d)) direction for a 40× air objective and 100× oil immersion objective. Solid line: 780 nm wavelength; Dotted line: 780 nm two photon process; Dashed line: 390 nm. The higher numerical aperture gives a much tighter beam waist. The ratio between axial and horizontal FWHM of the two-photon distribution is 3:1 for the oil immersion objective and 7:1 for the air objective. This agrees with the experimental results by us and others. On the square of the intensity makes sure that they do not occur in other regions, even when they are exposed for elongated time periods. Single photon polymerization does not take place since double the polymerization threshold wavelength is used. The resulting structures have high spatial resolution and sharp edges (Fig. 3 (a) and (d)).

The volume which is polymerized with a single exposure is a prolate spheroid with a long axis of 1.2 µm and a short axis of 0.29 µm. The shape of this volume poses a lower limit for sharp feature and small structure sizes. Nevertheless, by overlapping subsequent layers, features much finer than the size of the exposed volume can be achieved (Fig 5). Lemercier et al. tested different photoinitiator-monomer systems optimized for two-photon processes. With a 40× magnification, 0.75 NA objective, they obtained exposed spheroids with a length of 2.9 µm and width of 0.44 µm. The length to width ratio of 7 even surpasses the here observed ratio of 4. These high ratios are caused by the much steeper intensity gradient in X and Y direction compared to Z.

The intensity profiles in the focal spot of a 40× and 100× objective for 390 nm, 780 nm and a 780 nm two photon process are shown in Fig. 7. They were obtained by numerical calculation of the electrodynamic field using multipole expansion of the incoming Gaussian laser beam and a point matching algorithm. The dimensions of the theoretical diffraction limited spot for the two photon process agree well with the here observed dimensions of the polymerized region. For the 1.3 NA objective, the theoretical ratio of axial to horizontal extent of 3:1 is very close to the observed ratio of 4:1. For the 0.65 NA objective, the theoretical ratio is dramatically increased.
to 7:1, which agrees very well with the ratio obtained from Lemercier et al.\textsuperscript{5} Although the intensity threshold for the onset of polymerization is not exactly known for the systems discussed here, one finds that the full width half maximum (FWHM) values of the theoretical intensity curves are very close to the experimentally observed dimensions of the photopolymerized voxels.

Ormos et al. achieve a line width on 0.5 \( \mu \)m with a laser power of 20 mW at 532 nm wavelength.\textsuperscript{2} To correct for the spot size of the polymerized region, Lim et al. have developed a contour offset algorithm which enhances the similarity of the fabricated structure with the original mask.\textsuperscript{7} They also investigate the growth of the polymerized region with exposure time and find that size changes below 100 ms but is relatively stable above 100 ms.

The size of the polymerized region strongly depends on the intensity profile in the focal spot. Even though the wavelength of 780 nm yields a larger diffraction limited spot, the polymerized region is comparable to a diffraction limited spot at half the wavelength (390 nm) (Fig. 7, Ref.\textsuperscript{8}).

The experimental setup we use has several advantages. Scanning the stage instead of the laser beam makes sure that exposure is unchanged over the whole process. Scanning the beam can cause the filling of the objective back aperture to change which results in changes in intensity and focal spot size. Also, the size range of the structure is quite limited. Growing the structure upside down, meaning on a second coverslip as illustrated in Fig. 2, has two main advantages: First, growing can start at the cover slip. Thus, deformation of the structure by hydrodynamic forces as observed by Qi et al. does not occur, since all structures are attached.\textsuperscript{9} Second, the laser beam has never to pass through an already polymerized structure and will not be deflected. It is claimed that the change of the refractive index during polymerization is too small to cause any deflection.\textsuperscript{3} Nevertheless, structures during polymerization can be clearly seen in the bright field microscope, suggesting that the change in refractive index is not negligible.

A further advantage of our system is the use of \textit{in situ} development. Up to now, structures have been either produced on a stalk fixed to the slide and are not movable\textsuperscript{3,10} or many structures were produced and one structure was eventually captured in an optical trap.\textsuperscript{2,11,12} We produce only one single structure and trap it during the development process. The microfluidic device also offers the possibility to exchange the developing agent (here acetone) with water or biocompatible medium.

Compared to other micromachine element fabrication techniques, like electron beam lithography,\textsuperscript{4} two photon polymerization offers the advantage that all steps can be performed under the same microscope, and a new component can be designed and produced within an hour. If the requirement for a new mechanical component should arise during a biophysical experiment, it could immediately be addressed and a solution realized.

5. SUMMARY

In conclusion, we have presented our method of performing two-photon polymerization and have characterized the resulting three dimensional structures. \textit{In situ} development adds further advantages to the method. We aspire to 3D trap rotating structures in biocompatible environments. So far, only structures fixed to the coverslip have been used in these environments, rendering them useless for the investigation of a cell located at another site on the coverslip. Free floating structures were only trapped in acetone up to now.

Acknowledgements

The support of the Centre for Microscopy and Microanalysis (CMM) of The University of Queensland, especially of Dr. Kim Sewell, is greatly acknowledged. We further acknowledge the contributions of Dr. Wolfgang Singer and Vincent Loke. The research was also supported by the Graduate School Research Travel Award of The University of Queensland.

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