# A versatile diffractive maskless lithography for single-shot and serial microfabrication

Nathan J. Jenness, 1,2,\* Ryan T. Hill, Angus Hucknall, Ashutosh Chilkoti, and Robert L. Clark

<sup>1</sup>Center for Biologically Inspired Materials and Material Systems, Duke University, Durham, NC 27708, USA

<sup>2</sup>Hajim School of Engineering and Applied Sciences, University of Rochester, Rochester, NY 14627, USA

\*njenness@seas.rochester.edu

**Abstract:** We demonstrate a diffractive maskless lithographic system that is capable of rapidly performing both serial and single-shot micropatterning. Utilizing the diffractive properties of phase holograms displayed on a spatial light modulator, arbitrary intensity distributions were produced to form two and three dimensional micropatterns/structures in a variety of substrates. A straightforward graphical user interface was implemented to allow users to load templates and change patterning modes within the span of a few minutes. A minimum resolution of ~700 nm is demonstrated for both patterning modes, which compares favorably to the 232 nm resolution limit predicted by the Rayleigh criterion. The presented method is rapid and adaptable, allowing for the parallel fabrication of microstructures in photoresist as well as the fabrication of protein microstructures that retain functional activity.

©2010 Optical Society of America

**OCIS codes:** (140.3390) Laser materials processing; (350.3450) Laser-induced chemistry; (230.6120) Spatial light modulators; (050.6875) Three-dimensional fabrication.

#### References and Links

- 1. R. M. Guijt, and M. C. Breadmore, "Maskless photolithography using UV LEDs," Lab Chip 8(8), 1402–1404
- S. A. Lee, S. E. Chung, W. Park, S. H. Lee, and S. Kwon, "Three-dimensional fabrication of heterogeneous microstructures using soft membrane deformation and optofluidic maskless lithography," Lab Chip 9(12), 1670– 1675 (2009).
- T. Nisisako, and T. Torii, "Formation of biphasic Janus droplets in a microfabricated channel for the synthesis of shape-controlled polymer microparticles," Adv. Mater. 19(11), 1489–1493 (2007).
- A. Jayagopal, G. P. Stone, and F. R. Haselton, "Light-guided surface engineering for biomedical applications," Bioconjug. Chem. 19(3), 792–796 (2008).
- F. Zhang, R. J. Gates, V. S. Smentkowski, S. Natarajan, B. K. Gale, R. K. Watt, M. C. Asplund, and M. R. Linford, "Direct adsorption and detection of proteins, including ferritin, onto microlens array patterned bioarrays," J. Am. Chem. Soc. 129(30), 9252–9253 (2007).
- M. C. George, A. Mohroz, M. Piech, N. S. Bell, J. A. Lewis, and P. V. Braun, "Direct Laser Writing of Photoresponsive Colloids for Microscale Patterning of 3D Porous Structures," Adv. Mater. 21(1), 66–70 (2009).
- M. Campbell, D. N. Sharp, M. T. Harrison, R. G. Denning, and A. J. Turberfield, "Fabrication of photonic crystals for the visible spectrum by holographic lithography," Nature 404(6773), 53–56 (2000).
- 8. S. Jeon, V. Malyarchuk, J. A. Rogers, and G. P. Wiederrecht, "Fabricating three-dimensional nanostructures using two photon lithography in a single exposure step," Opt. Express 14(6), 2300–2308 (2006).
- J. H. Jang, C. K. Ullal, M. Maldovan, T. Gorishnyy, S. Kooi, C. Y. Koh, and E. L. Thomas, "3D micro- and nanostructures via interference lithography," Adv. Funct. Mater. 17(16), 3027–3041 (2007).
- K. Itoga, J. Kobayashi, M. Yamato, A. Kikuchi, and T. Okano, "Maskless liquid-crystal-display projection photolithography for improved design flexibility of cellular micropatterns," Biomaterials 27(15), 3005–3009 (2006)
- 11. T. Naiser, T. Mai, W. Michael, and A. Ott, "Versatile maskless microscope projection photolithography system and its application in light-directed fabrication of DNA microarrays," Rev. Sci. Instrum. 77(6), 063711 (2006).
- N. J. Jenness, K. D. Wulff, M. S. Johannes, M. J. Padgett, D. G. Cole, and R. L. Clark, "Three-dimensional parallel holographic micropatterning using a spatial light modulator," Opt. Express 16(20), 15942–15948 (2008).
- 13. S. Hasegawa, Y. Hayasaki, and N. Nishida, "Holographic femtosecond laser processing with multiplexed phase Fresnel lenses," Opt. Lett. **31**(11), 1705–1707 (2006).
- Y. Kuroiwa, N. Takeshima, Y. Narita, S. Tanaka, and K. Hirao, "Arbitrary micropatterning method in femtosecond laser microprocessing using diffractive optical elements," Opt. Express 12(9), 1908–1915 (2004).

- D. Gil, R. Menon, and H. I. Smith, "The case for diffractive optics in maskless lithography," J. Vac. Sci. Technol. B 21(6), 2810–2814 (2003).
- J. Amako, H. Miura, and T. Sonehara, "Speckle-Noise Reduction on Kinoform Reconstruction Using a Phase-Only Spatial Light-Modulator," Appl. Opt. 34(17), 3165–3171 (1995).
- 17. D. Dendukuri, D. C. Pregibon, J. Collins, T. A. Hatton, and P. S. Doyle, "Continuous-flow lithography for high-throughput microparticle synthesis," Nat. Mater. 5(5), 365–369 (2006).
- J. C. Love, D. B. Wolfe, H. O. Jacobs, and G. M. Whitesides, "Microscope projection photolithography for rapid prototyping of masters with micron-scale features for use in soft lithography," Langmuir 17(19), 6005–6012 (2001)
- D. W. Palmer and S. K. Decker, "Microscopic Circuit Fabrication on Refractory Superconducting Films," Rev. Sci. Instrum. 44(11), 1621–1624 (1973).
- 20. R. W. Gerchberg, and W. O. Saxton, "A practical algorithm for the determination of phase image and diffraction plane pictures," Optik (Stuttg.) 35, 237–248 (1972).
- J. Leach, K. Wulff, G. Sinclair, P. Jordan, J. Courtial, L. Thomson, G. Gibson, K. Karunwi, J. Cooper, Z. J. Laczik, and M. Padgett, "Interactive approach to optical tweezers control," Appl. Opt. 45(5), 897–903 (2006).
- R. Nielson, B. Kaehr, and J. B. Shear, "Microreplication and design of biological architectures using dynamic-mask multiphoton lithography," Small 5(1), 120–125 (2009).
- F. L. Yap and Y. Zhang, "Protein and cell micropatterning and its integration with micro/nanoparticles assembly," Biosens. Bioelectron. 22(6), 775–788 (2007).
- S. Basu and P. J. Campagnola, "Properties of crosslinked protein matrices for tissue engineering applications synthesized by multiphoton excitation," J. Biomed. Mater. Res. 71A(2), 359–368 (2004).
- R. P. Ekins, "Ligand assays: from electrophoresis to miniaturized microarrays," Clin. Chem. 44(9), 2015–2030 (1998).
- M. A. Holden and P. S. Cremer, "Light activated patterning of dye-labeled molecules on surfaces," J. Am. Chem. Soc. 125(27), 8074–8075 (2003).
- B. Kaehr, N. Ertas, R. Nielson, R. Allen, R. T. Hill, M. Plenert, and J. B. Shear, "Direct-write fabrication of functional protein matrixes using a low-cost Q-switched laser," Anal. Chem. 78(9), 3198–3202 (2006).

## 1. Introduction

Microfabrication via wavefront modulation has several key advantages that could prove useful for the development of many lab-on-a-chip technologies. Already recognized for high efficiency and flexible pattern generation, phase holograms also allow for the direct integration of arbitrary micropatterning into microscale devices and structures. These features lend themselves well to working with a wide range of device geometries and immobilizing target materials *in situ* when desired. Several researchers have recently described *in situ* photolithography and photocrosslinking for spatially defined fabrication inside microfluidic channels [1–3], biosensors [4,5], and colloidal suspensions [6].

A hindrance to the widespread adoption of laser direct-write processes is the dichotomy between the large quantity of spatial coordinates required for fabrication and the inherent serial nature of the processes, which limits throughput and adds complexity. To circumvent these limitations, significant effort has been devoted to increasing the speed of serial scanning and leveraging parallel processing methods. Towards this end interference lithography has emerged as a method to generate periodic 3D micro- and nanoscale structures over millimeter scale areas [7–9]. However, interference holography is limited to patterning homogenous arrays of generally crystalline structures and does not allow the fabrication of varying microstructures of arbitrary shape on a substrate, a capability that would be useful for many applications.

One solution to this problem involves the use of addressable optical elements to display digital photomasks or phase holograms. Digital photomasks directly modify the amplitude profile of light to project and transfer arbitrary patterns into target materials [10,11], but limit structures to geometries extruded from the 2D photomask patterns. By modifying the phase of light instead of the amplitude, phase holograms not only provide greater efficiency but also allow the parallel fabrication of arbitrary 3D microstructures through the simultaneous generation of multiple foci [12–15]. Even though the manipulation of several foci using phase holograms enables parallel direct-write fabrication, a purely phase-modulated single-shot mode is desirable to match the current capabilities of optical projection and mask-based photolithographic methods.

The inability of holographic maskless lithography to operate in a single-shot mode can be attributed to the presence of speckle noise. Caused by the interference of complex amplitude

fields due to phase irregularities in holograms, speckle noise produces areas of zero intensity within otherwise continuous patterns [16]. These areas of zero intensity result in unexposed regions within a target material producing flawed and disjointed structures. A method that incorporates a speckle correction to provide seamless single-shot maskless microfabrication is necessary for a wide variety of high-throughput and geometrically specific applications.

In addition to improving parallel processing capability by providing a multi-foci and single-shot mode, phase holograms can also create and direct intensity distributions into multiple planes. This level of control surpasses that of optical projection lithography (OPL) which can only create patterns within a single projection plane [10,11,17–19]. The need to precisely control the aspect ratio of 3D microstructures has led to the development of optofluidic maskless lithography (OFML). During OFML, a height-tunable microfluidic channel controls the depth of the fabrication area enabling layer-by-layer 3D fabrication [2]. While this method improves upon OPL through the addition of a third dimension of spatial control, it does not provide complete 3D spatial control because microstructures with void spaces indicative of bridges or tunnels cannot be fabricated. The addition of this level of control over the morphology of microstructures will open up new applications not only in microfluidics but also for the fabrication of optical and biomolecular devices.

Herein, we combine the desirable traits of several lithographic processes into a single dynamic, maskless, and holographic process that is capable of rapid 3D microfabrication. In this approach, laser light is directed into arbitrary intensity distributions and multiple planes via phase holograms displayed on a spatial light modulator (SLM). The computer-generated phase holograms contain all the spatial information required for 3D microfabrication, eliminating the need for programmable positioning stages. We demonstrate rapid prototyping, with multiple 2D and 3D pattern templates, in photoresists, photopolymers, and proteins through serial and single-shot lithography modes. The methodology described herein distinguishes itself from previous methodologies used to address microscopic regions, in that it can readily and rapidly be interchanged from serial to single-shot mode. The availability of this option, coupled with the ability to perform 3D patterning of arbitrary features, provides greater flexibility for the fabrication or modification of lab-on-a-chip devices.

## 2. Experimental

## 2.1 Optical system

The 532 nm output from a Q-switched neodymium-doped yttrium vanadate (Nd:YVO<sub>4</sub>) laser (Coherent PRISMA-532-12-V) was polarized by a 1/2 wave plate and aligned into a two-component series of lenses for expansion and collimation (Fig. 1). The lenses were selected such that the expanded beam would slightly overfill the active portion of the SLM display (HOLOEYE LC-R 2500). The display surface of the SLM contains a  $1024 \times 768$  array of individually addressed 19  $\mu$ m liquid crystals positioned in front of reflective silicon pixels. The refractive index of the liquid crystals above each pixel is modified by applying a voltage, which adjusts the phase of incoming plane wavelets to create the desired phase delays dictated by a  $512 \times 512$  phase hologram. A properly selected polarization ensures maximum energy transfer during phase modulation, while a slight overfill ensures an even distribution of light over the active pixels in the SLM.

Only the first diffraction order was used for patterning, so unwanted diffraction orders were removed by spatial filtering. Using a series of lenses and mirrors the phase modulated light from the SLM was directed into the epi-fluorescence port of the inverted microscope (Zeiss Axiovert 200). A dichroic beam splitter enabled real-time CCD (Hamamatsu C2400) monitoring of the sample surface (a 610 nm long pass glass filter prevents camera saturation) and directed the laser beam into the specimen plane through a high numerical aperture (NA) objective (Zeiss Plan-Apochromat 100x/1.4 NA). The objective is designed for DIC microscopy, so the polarization of the laser was maintained. A second objective (Zeiss Plan-Neofluar 10x/0.3 NA) was also used in order to create larger scale micropatterns. The field of view (FOV) visible on the CCD limited the patterning area available for each objective. The

visible region for the 100x objective was  $56 \mu m x 42 \mu m$ , while that of the 10x objective was 0.56 mm x 0.42 mm. A positioning stage was used to move between individual patterning areas on a sample.

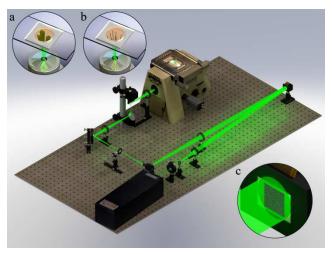


Fig. 1. The SLM-based diffractive patterning system. The output from a Q-switched 532 nm laser is polarized, collimated, and expanded to address the display of a SLM operating in phase-only mode. Three relay lenses and several mirrors direct the SLM modulated beam to the pupil plane of a high NA microscope objective. (a) Close up of an intensity distribution generated for single-shot processing, three shapes are projected into a sample material at once. (b) An intensity distribution of nine foci generated for the simultaneous serial processing of nine unique structures. (c) Close up of the SLM display showing the expanded, collimated beam incident upon a phase hologram. The phase hologram can be modified to produce any desired intensity distribution including those seen in (a) and (b).

## 2.2 Hologram generation

The computer-generated holograms required for phase modulation are produced based upon user-defined pattern templates. The template area is 512 x 512 pixels to match the resolution of the hologram displayed on the SLM. The choice of image format (bitmap, jpeg, or tiff) appears to have no measureable impact on the final system resolution. The 2D single-shot templates can be designed with standard imaging processing software, while the serial templates and 3D single-shot templates are designed in the computer aided drafting software SolidWorks. SolidWorks allows for the creation of stereolithography (STL) files that generate a set of 3D spatial coordinates. The information in the STL file is divided into individual points for serial patterning or individual layers for single-shot patterning. Then holograms are created for each point or layer and combined for display on the SLM using the Gerchberg-Saxton algorithm.

The Gerchberg-Saxton algorithm is an iterative Fourier-transform-based algorithm that calculates the phase required at the hologram plane to produce the predefined intensity distribution at the focal plane. The algorithm quickly converges, after completing a few iterations, producing the desired phase. Because beam shaping is limited to the focal plane, however, only 2D intensity patterns can be generated. A third dimension of control is obtained through the addition of a lens phase, which provides axial positioning of the intensity patterns within the sample. Minimum feature sizes using this technique are submicron in the radial dimensions and low micron in the axial dimension.

# 2.3 Fabrication

For the initial single-shot fabrication, a 1.5  $\mu$ m thick layer of Microchem S-1813 photoresist was applied to a no. 1 cover glass (VWR). The layer was formed by spin coating at 500 rpm for 5 s with and then at 3500 rpm for 40 s with a ramp rate of 300 rpm. The substrate was then

prebaked at 115°C for 60 s. After exposure, the sample was developed for 60 s using MF-319 developer and dried using nitrogen gas.

For 3D serial patterning, both Norland 63 photopolymer and Microchem SU-8 2010 negative tone photoresist were used. For structures created using Norland 63, a small droplet of the photopolymer was deposited on a glass cover slip. After exposure, non-modified photopolymer was removed by washing with pure methanol. Nitrogen gas was used to dry the sample after washing. For structures created using Microchem SU-8 2010, a 20 µm thick layer of the photoresist was formed by spin coating at 500 rpm for 5 s and then at 1000 rpm for 30 s with a ramp rate of 300 rpm. The substrate was then baked for 1 min at 65°C and 3 min at 95°C. After exposure the sample was baked for 1 min at 65°C and 2 min at 95°C. The sample was then developed for 3 min using SU-8 developer and dried using nitrogen gas.

While the Microchem S-1813, Norland 63, and Microchem SU-8 2010 are designed for near UV exposure they all have some photosensitivity at the 532 nm wavelength. To account for the decreased photosensitivity, a larger energy dose was required to induce the chemical changes necessary for structure formation.

Protein structures were fabricated in 20 µL sample wells on a no. 1 cover glass. The wells were made by punching 3.0 mm diameter holes in 1 mm thick PDMS films and then pressing the films onto the cover glass. A 200 mg mL<sup>-1</sup> solution was formed by dissolving 40 mg of FITC conjugated BSA (Sigma Aldrich A9771) in 200 µL of 1x phosphate buffered saline (PBS). The BSA and PBS were placed briefly in a microcentrifuge set to 5000 rpm to aid in the dissolution process. Next, a 33 mg mL<sup>-1</sup> solution was made by dissolving 2 mg of FITC conjugated avidin (Rockland A003-02) in 60 µL of 1x PBS. Again the microcentrifuge was used to aid the dissolution process. A 9:1 by volume solution of the BSA and avidin was made, yielding a total protein concentration of 183 mg mL<sup>-1</sup>. An 8 μL aliquot of the solution was pipetted into a PDMS well. The well was positioned in the focal plane of the objective for fabrication. After exposure the samples were rinsed five times using the 1x PBS and then left immersed in 1 µM ATTO 650-biotin (ATTO-TEC) for 20 min. The sample was then rinsed with PBS five times and left immersed in PBS for fluorescent imaging using an epifluorescence microscope (Nikon TE2000U). A B-2E/C blue excitation (465 - 496 nm) filter set was used to excite the FITC conjugated proteins, as the FITC absorbs blue light (495 nm) and emits green light (528 nm). A Cy5 filter set was used for red excitation (590 - 650 nm) as the ATTO 655-biotin absorbs light at 655 nm and emits red light (715 nm).

# 2.4 Scanning electron microscope (SEM)

All samples were prepared for the SEM by sputter coating a 30 nm nominal layer of Au over the entire sample surface. A FEI XL30 SEM operating in secondary electron imaging mode was used to view and catalog the final microstructures.

For protein structures an addition desiccation step was conducted prior to sputter coating. The desiccation was performed by the sequential immersion (10 min per solution) of the protein sample in 2:1 ethanol/deionized water, ethanol, ethanol, 1:1 ethanol/methanol, and methanol. The samples were then allowed to air dry for several hours.

### 3. Results and discussion

Diffractive maskless lithography uses computer-generated holograms that are displayed on a phase-only SLM to shape and distribute coherent laser light in three dimensions. As shown in Fig. 1, the SLM is positioned at a focal plane conjugate to the pupil plane (back focal plane) of a high numerical aperture (NA) microscope objective. The phase distributions displayed on the SLM, commonly termed phase holograms, are derived from the Gerchberg-Saxton algorithm (GSA) [20] and generate 2D light distributions by adjusting the phase of a uniform wavefront. The Fourier transforming property of a lens converts the angular information contained within the modified wavefront into the desired spatial distribution(s) at the focal plane. Figure 1 illustrates the conversion of a phase hologram derived from a target template into an intensity distribution at the focal plane of the system. Similar to projection methods

this distribution can be used to perform lithography. However, while the intensity distribution reflects an accurate representation of the target image, discontinuities comprised of zero intensity result from the presence of speckle noise. A time-averaged corrective technique [16] previously used in photographic films was modified and implemented to improve pattern continuity for the single-shot patterning mode.

In order to perform single-shot lithography, several phase holograms containing the phase information for a desired image were calculated and displayed in succession. The stochastic nature of the phase error introduced during the calculation of each phase hologram, which causes speckle noise, was averaged by the exposure of a region to multiple iterations of the same computer-generated hologram. The fidelity of the final pattern can be expressed in terms of the speckle contrast,

$$C = \frac{\sigma}{\langle I \rangle} = \frac{1}{\sqrt{N}} \tag{1}$$

where  $\sigma$  is the standard deviation,  $\langle I \rangle$  is the average intensity, and N is the number of hologram additions. Equation (1) reveals that the image contrast can be reduced by increasing the number of hologram additions. The single-shot capabilities of the system were evaluated by varying the N value within a preset exposure time of 10 s. Several N values were selected to understand the trade-off between increased image fidelity and hologram calculation time. A significant improvement in overall image fidelity was realized using as few as 10 holograms, C = 0.32. Further evaluation suggested that an exposure containing 30 holograms (C = 0.18), requiring less than 35 seconds to calculate, provided highly resolved and continuous patterns.

The versatility of the maskless single-shot mode was demonstrated by the fabrication of patterns within S-1813 positive photoresist. A diverse range of arbitrary templates were designed and patterned on a single sample within 10 minutes (Fig. 2) using single-shot holographic processing. The templates can be designed with any image processing software capable of creating bitmaps, jpegs, or tiffs. Resolutions of approximately 700 nm are achieved in patterns that contain features with at least one single-pixel dimension [Fig. 2(e)]. The Rayleigh criterion predicts a minimum resolution of 232 nm for the system (532 nm wavelength, 1.4 NA); however, the non-ideal properties of the SLM, including a 19 µm pixel pitch and 93% fill factor, likely account for this discrepancy in resolution. Nevertheless, these results indicate that the phase-based single-shot mode is capable of providing resolution and pattern continuity comparable to many amplitude-based OPL methods.

The parallel processing capabilities of the system were also demonstrated using S-1813 resist. A user-defined feature was created and discretized into pixel coordinates. Next, individual holograms containing phase information for an array of foci were generated for each of these coordinates. User-defined spacing and feature size, along with the FOV of the microscope objective, dictate the number of features/foci in the final multi-component pattern. When displayed on the SLM, each phase hologram produces an intensity distribution containing the specified number of arrayed foci. Because the SLM has a frame rate of 72 Hz, the holograms may be combined and displayed sequentially as a movie for serial patterning. The serial fabrication of a 25-feature array of squares [Fig. 2(f)] using this methodology took less than a minute.

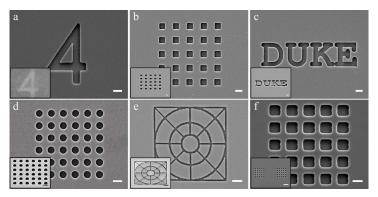


Fig. 2. SEM images of various 2D patterns fabricated using the described method with a 100x objective. The inset SEM images captured at a 26° angle to the sample surface show the photoresist depth. The patterns in (a-e) were fabricated using the single-shot mode, while the pattern in (f) was fabricated in serial mode. As shown in movie [(a) Media 1], single-shot patterns required the display of 30 phase holograms, during a 10 second fabrication time at an average laser power of ~1 mW, to eliminate speckle and produce smooth continuous features. The arrays and arbitrary shapes demonstrate the versatility of the single-shot method. The array in (f) shows the ability to provide high-throughput processing in serial mode, while the inset displays replication accuracy. The phase holograms were displayed on the SLM at 8 fps and created 25 independent foci to simultaneously transfer each feature of the 5 x 5 array. An average power of 1 mW was used. Scale bars are 5 $\mu$ m except the inset of (f) which is 20  $\mu$ m.

Having demonstrated the versatility of the 2D serial and single-shot modes, we next attempted fabrication of 3D microstructures. Because the linear absorption characteristics of the S-1813 at the 532 nm laser wavelength preclude 3D fabrication, a commercially available photopolymer (Norland 63) was used. The absorption properties of the polymer and the nonlinear excitation provided by the laser enable the 3D positioning of voxels. A specially calculated lens phase [21] was added to each hologram to shift 2D distributions into arbitrary planes. The axial shifts required for a layer-by-layer 3D fabrication approach were determined by the depth of focus (DOF) within the target material. The DOF in the photopolymer, which was experimentally determined to be ~5 µm, dictated the axial resolution during fabrication. However, the axial resolution could be improved when fabricating solid structures without voids by adjusting the axial shifts such that the layers overlap. Figures 3(a) through 3(d) show nine different microstructures that were simultaneously fabricated using multiple foci. These results demonstrate 3D control over the morphology of the fabricated structures as well as the ability to simultaneously fabricate structures with different shapes on the same substrate using a single phase-modulated source beam. We believe that the ability to simultaneously fabricate 3D microstructures of different shapes is a unique feature of this methodology, as we are not aware of another method that provides this capability.

To further illustrate our 3D fabrication capability, we used the negative tone photoresist SU-8 2010 because the thermal and chemical properties of this epoxy-based resist are better suited for higher aspect ratio micropatterning than the Norland 63. Figures 3(f) through 3(h) display SEM micrographs of a microscale bridge that was serially fabricated using two focal points directed by phase holograms derived from a predesigned template [Fig. 3(e)]. We chose this microstructure to demonstrate the ability of diffractive maskless lithography to fabricate microstructures that contain voids. Because the system utilizes an inverted microscope, the voids beneath the rails demonstrate true 3D fabrication, as the laser light can be focused through areas of the resist leaving them unaltered. This level of spatial control is akin to the many multi-photon systems presented in the literature, but does not require the use of any moving components, such as micropositioning stages.

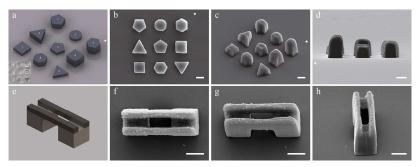


Fig. 3. SEM micrographs of 3D microstructures fabricated using the serial processing mode. (a) The 9-feature pattern was designed in CAD software and then all features were simultaneously fabricated in NOA 63 using a single phase-modulated source beam (Media 2). The various views (b-d) show the morphology of the pattern. Features 2, 5, 7, & 9 are 5  $\mu m$  in height, features 1, 4, & 8 are 7.5  $\mu m$  in height, and features 3 & 6 are 10  $\mu m$  in height. The stars in each image indicate the pattern orientation. The holograms were displayed on the SLM at 8 fps with an average power of 62 mW through a 100x objective. (e-h) A bridge type structure fabricated in SU-8 2010 demonstrates complete 3D control of fabrication, including the ability to create voids within the microstructure. Holograms were displayed on the SLM at 2 fps with an average power of 62 mW through the 100x objective. The scale bars in all images are 5  $\mu m$ .

The noncontact fabrication afforded by diffractive maskless lithography is well suited for the processing of substrates immersed in an aqueous environment, which makes it an attractive method for creating proteinaceous microstructures. Recently, microstructures composed of proteins have been utilized to direct cell motility [22], detect biological molecules [23], and as scaffolds for tissue engineering applications [24]. Towards the goal of creating biosensors based on the ambient analyte theory [25], we next explored the single-shot fabrication of protein features on the order of tens of microns. A biosensor of this size satisfies the ambient analyte theory because it performs detection of a target in solution such that the equilibrium concentration of the test sample is unaffected. Patterns were created on a glass surface from bovine serum albumin (BSA) conjugated with fluorescein isothiocyanate (FITC) contained within a 20 µL sample well. A three-shape template was transferred to the BSA protein using a time averaged single-shot exposure [Fig. 4(a)]. The subsequent fluorescent image [Fig. 4(b)] and SEM micrograph [Fig. 4(c)] indicate the successful fabrication of active microstructures. We believe that the formation of microstructures that are stable in an aqueous environment is due to the photocrosslinking of the protein within the exposure volume mediated by the conjugated FITC, as previously reported by Cremer, Shear, and associates [26,27]. Next, to conclusively demonstrate that protein activity is retained after the photocrosslinking of protein microstructures, the retention of biotin binding was tested using a FITC-BSA solution spiked with FITC-avidin. The BSA/avidin structures shown in Fig. 4(d) were incubated with a fluorescently labeled biotin solution and then thoroughly rinsed to remove any unbound biotin. The fluorescent images in Figs. 4(e) and 4(f) indicate the presence of bound biotin only within the boundaries of the BSA/avidin features. This result clearly demonstrates that protein structures microfabricated by dynamic maskless lithography retain the ability to bind their ligand in an aqueous environment, suggesting that this microfabrication methodology will allow the fabrication of functionally active microstructures of other ligand-binding proteins and enzymes with micron-level spatial resolution.

At this point, it is important to point out that the lithographic technique is not limited to operating with only the 532 nm wavelength. While the versatility to fabricate structures in the particular photoresists, photopolymers, and proteins available to our group governed the choice of this wavelength, the SLM can accommodate wavelengths ranging from 400 to 700 nm. By simply replacing the source laser, the system can be tailored to fabricate structures in a variety of photoactive materials.

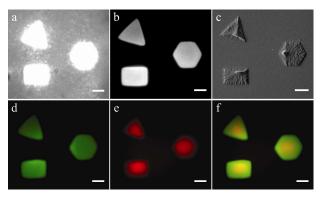


Fig. 4. BSA-FITC solution exposed to three time-averaged patterns simultaneously at 110 mW for 10 s. (a) Real-time microscope image captured during fabrication. (b) Fluorescent image of patterned protein after sample wash with 1x PBS. (c) SEM image of sample after a chemical desiccation process. Predicted feature dimensions were assessed using area measurements from both the fluorescent and SEM images. The fluorescent measurements indicated the features were ~6% larger than desired, while the SEM measurements indicated they were ~6% smaller than desired; demonstrating an accurate tolerance for pattern transfer. Next, a BSA-FITC solution spiked with avidin conjugated FITC was patterned. Biotin conjugated with ATTO 655 was applied to the resulting BSA/avidin structures for 20 min to assess the retention of avidin binding ability. Fluorescent microscope images were taken using (d) FITC and (e) Cy5 filter sets. A combined fluorescent image (f) reveals the functionality of the substrate after patterning. All scale bars are 50  $\mu m$ .

## 4. Conclusions

We have demonstrated a diffractive, dynamic, and maskless lithographic technique for the rapid 2D and 3D fabrication of microstructures. Using computer-generated holograms to modulate coherent wavefronts, we perform both serial and single-shot lithography with submicron resolution; features that we believe are unique to this microfabrication methodology. In addition, the use of phase manipulation allows us to eliminate the requirement for a micropositioning platform and provides the unique ability to pattern several unique 3D structures in a parallel fashion. To our knowledge, it is the only system through which the user has simultaneous and independent control over the morphology of each structure in both the lateral and axial dimensions. The versatility of the system was further illustrated by the rapid design and transfer of pattern templates onto a variety of materials, including proteins. We believe that further refinement of spatial controls and implementation of advanced optical aberration correction will result in improved resolution and replication accuracy, aiding in the fabrication and modification of many optical and lab-on-a-chip devices.

## Acknowledgements

This research was supported by funding from National Science Foundation grants CMMI-0609265 (R. L. C.) and IGERT Fellowship DGE-0221632 (N. J. J.); and National Institute of Health grant 1R21EB009862 (A. C.).