Hybrid Laser Printing

# Hybrid Laser Printing of 3D, Multiscale, Multimaterial Hydrogel Structures

Puskal Kunwar, Zheng Xiong, Yin Zhu, Haiyan Li, Alex Filip, and Pranav Soman\*

Fabrication of multiscale, multimaterial 3D structures at high resolution is difficult using current technologies. This is especially significant when working with mechanically weak hydrogels. Here, a new hybrid laser printing (HLP) technology is reported to print complex, multiscale, multimaterial, 3D hydrogel structures with microscale resolution. This technique utilizes sequential additive and subtractive modes of fabrication, that are typically considered as mutually exclusive due to differences in their material processing conditions. Further, compared to current laser writing systems that enforce stringent processing depth limits, HLP is shown to fabricate structures at any depth inside the material. As a proof-of-principle, a Mayan pyramid with embedded cube frame is printed using synthetic polyethylene glycol diacrylate (PEGDA) hydrogel. Printing of ready-to-use open-well chips with embedded microchannels is also demonstrated using PEGDA and gelatin methacrylate (GelMA) hydrogels for potential applications in biomedical sciences. Next, HLP is used in additive-additive modes to print multiscale 3D structures spanning in size from centimeter to micrometers within minutes, which is followed by printing of 3D, multimaterial, multiscale structures using this technology. Overall, this work demonstrates that HLP's fabrication versatility can potentially offer a unique opportunity for a range of applications in optics and photonics, biomedical sciences, microfluidics, etc.

### 1. Introduction

Over the course of billions of years, nature has created and refined numerous elegant biosynthetic processes to make sophisticated functional structures. In contrast, current manufacturing techniques are still limited in their ability to fabricate 3D, multiscale, multimaterial structures. New advances in fabrication methods are expected to revolutionize the development of next-generation devices for optics, photonics, microfluidics, soft robotics, flexible electronics, organ-on-a-chip, and many other applications.<sup>[1–3]</sup> Already, the emergence of additive manufacturing methods such as extrusion-based fused deposition

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modeling (FDM), inkjet-based multiJet modeling (MIM), digital light processing (DLP), and stereolithography (SLA), have allowed the fabrication of structures and devices with high design flexibility, in a highly automated, assembly-free, and low cost manner.<sup>[2,4]</sup> A new version of DLP, coined as continuous liquid interface production (CLIP), is able to fabricate centimeter-sized polymeric constructs in a quick and continuous fashion.<sup>[5]</sup> However, these methods cannot fabricate structures with resolution better than 25  $\mu$ m. Multiphoton polymerization (MPP), often coined as direct laser writing, is an additive manufacturing method that allows the printing of complicated 3D structures at sub-micrometer resolution.<sup>[6,7]</sup> However, slow point-by-point scanning approach generally makes this method impractical for printing centimeter scale multiscale structures.

As compared to most of the additive methods discussed above, subtractive manufacturing methods based on lithography provide similar resolution for fabricating micro- and nano-scale structures, but these methods are typically limited

to planar or 2.5D structures.<sup>[8]</sup> Multiphoton ablation (MPA), a method that utilize ultrafast lasers to remove material, is capable of creating embedded hollow microfeatures such as channels, grooves in glass and polymer substrates.<sup>[9,10]</sup> Similar to MPP, MPA also suffers from low scalability and throughput due to the serial nature of fabrication. Additionally, the dependence of MPA on the optical transparency of the material, limits its penetration depth and overall design flexibility. Few research groups have utilized the ability of ultrafast lasers to process material in both additive–subtractive method.<sup>[10–12]</sup> But, current laser-based hybrid methods are limited by scalability, types of specialized materials, and incompatible laser processing requirements, thereby preventing its widespread use in the field.

In this work, we report the design and development of a hybrid laser printing (HLP) technology, that combines the key advantages of CLIP process (quick on-demand continuous fabrication) and MPP and MPA processes (high-resolution and superior design flexibility). Using a series of proof-of-principle experiments, we show that HLP is capable of printing 3D, multiscale, multimaterial structures using model biocompatible





hydrogel materials that are highly difficult and/or extremely time consuming to fabricate using current technologies. We demonstrate the HLP versatility of printing in three different modes of fabrication: additive/subtractive, additive/additive and multimaterial modes and we foresee that HLP's fabrication capabilites can be applied to a broad range of applications.

### 2. Design of Hybrid Laser Printer

HLP setup consists of a femtosecond laser source (Coherent, Ti:Sapphire), which is used in both additive and subtractive modes of fabrication. The additive cross-linking mode, indicated by solid blue arrows in **Figure 1**, is created by passing the fs-laser beam through a second harmonic generator (SHG) to obtain ultraviolet wavelengths and then spatially modulated via a digital micromirror device (DMD). DMD is an electronic board embedded with an array of micromirrors. Based on a

user-defined image, DMD can selectively switch mirrors into either an ON state or an OFF state and create a light pattern that selectively cross-links photosensitive prepolymer into 2D layers of a defined thickness. The subtractive ablation mode, indicated by the dashed red arrows in Figure 1, utilizes fs-laser beam directed via an objective lens to ablate voids within the previously cross-linked layer using MPA. Additionally, fs-laser can be also used in additive mode to polymerize 3D structures with microscale resolution using MPP. In this prototype, the additive mode can print ≈1 cm<sup>2</sup> feature in XY with smallest feature size of 30 µm, while the subtractive MPA mode can fully ablate features with a minimum feature size of 3 µm, based on the absorption properties of the hydrogel prepolymer. Figure 1 inset (big dashed box) shows the one sequence of the process flow of additive DMD cross-linking (CLIP) and subtractive MPA. Figure 1 inset (small dashed box) shows the schematic diagram of sample holder, stage, and Teflon window. A detail description of the optical setup of HLP and a photograph and



**Figure 1.** Schematic of femtosecond laser-based additive-subtractive/additive-additive HLP machine. [FS—femtosecond laser, SHG—second harmonic generator, I—isolator, BS—beam splitter, P—polarizer, HWP—half waveplate, SHT—shutter, L—lens, PH—pin hole, FM—mirror in a flip mount, D—diffuser, DMD—digital micromirror device, DM—dichroic mirror, CM—camera, OL—objective, S—sample, SH—sample holder, ST—stage, TF—teflon film]. Small dashed box: Schematic showing sample holder (SH) with teflon film (TF) and elevating stage (ST). Enlarged dashed box: Process flow of HLP showing one sequence of additive cross-linking and subtractive ablation steps.

detail description of the sample holder and elevating stage is described in the Experimental Section and Figure S1 in the Supporting Information.

# 3. Additive/Subtractive Mode of Hybrid Laser Printer

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We characterized the dead zone and ablation z-range of Hybrid laser printer. Fundamental to the HLP process is the relationship between the "dead zone" from the additive CLIP mode, and "ablation z-range" from subtractive MPA mode (Figure 2A). Characterization of dead zone is necessary for accurate determination of speed and z-resolution during the additive step while assessment of ablation z-range is important to determine the exact location of laser focus during the subtractive mode. Dead zone is a thin uncross-linked prepolymer solution between the Teflon window and the cross-linked hydrogel structure attached to the stage. This zone is created due to the inhibition of the photo-cross-linking process, a result of diffusion of oxygen through the permeable Teflon window. This prevents the adhesion of newly cross-linked layers to the bottom window and the liquid hydrogel prepolymer solution can freely refill the fabrication area adjacent to the Teflon window. The dead zone thickness can vary in the order of tens of micrometers depending upon the control parameter such as laser intensity, exposure time, cross-linking properties of hydrogel, and local oxygen concentrations. Furthermore, the dead zone defines the relationship between print speed and part resolution in the additive mode, and influences the linewidth of ablation part and penetration depths in the subtractive fabrication mode.

"Ablation z-range" is the region within the cross-linked hydrogel layers where material can be reliably removed in the subtractive ablation mode of HLP (Figure 2A). The two-photon absorption induced ablation process is limited to certain depth due to light absorption and scattering effects within the hydrogel, that defines the upper end of the "ablation z range." The lower end of the "ablation z-range" is the region just above the dead zone within the cross-linked hydrogel layer. Operating in this z-range ensures that the prepolymer solution does not inadvertently cross-link during the subtractive ablation step and avoids forming unwanted bubbles.

For this work, PEGDA prepolymer (90% by weight (wt), 700 MW) mixed with water soluble photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP 1% by wt) was chosen as a model synthetic hydrogel prepolymer solution. Wavelengths of 400 and 800 nm were chosen for additive crosslinking ( $\lambda_{add}$ ) and subtractive ablation ( $\lambda_{sub}$ ) modes respectively. In order to estimate the dead zone, the L shaped stage was placed exactly at 200  $\mu$ m from the surface of the Teflon window. A spatially modulated laser beam ( $\lambda_{add} = 400$  nm) was used to cross-link a uniform square shaped structures for a range of cross-linking laser power ( $P_{add}$ ) and exposure time ( $t_{add}$ ). The thickness of the structures was measured using HIROX microscopy and was subtracted from 200 µm to obtain the dead-zone thickness as a function of  $P_{add}$  and  $t_{add}$ . (Figure 2B). For the laser dosage used in this work, the dead-zone thickness ranges from  $8 \pm 8$  to  $97 \pm 3.5 \,\mu\text{m}$  and show an increasing trend with decreasing laser dosage. Increase in dead-zone thickness essentially means that a higher speed can be implemented to fabricate structures.

To measure the ablation z-range, width of the ablated lines was characterized at different depths of cross-linked structure (Figure 2C). First PEGDA slab was cross-linked using crosslinking laser power of  $(P_{add})$  of 150 mW and exposure time  $(t_{add})$  of 20 s that results in small dead zone of 30  $\mu$ m. Next, lines were ablated within the cross-linked structure using the ablation laser powers of  $(P_{sub})$  of 800, 1000, and 1200 mW, and scanning speed ( $\nu_{sub}$ ) of 100  $\mu$ m s<sup>-1</sup>. The ablated lines were written at the depth of 40-160 µm from the surface of Teflon window. Results demonstrate a decrease in linewidth with an increase in laser penetration depth, due to light absorption and scattering within the sample for all the laser powers. For  $P_{sub}$ of 800, 1000, and 1200 mW, the upper ends of ablated z-range were measured to be 120, 140, and 160 µm respectively. The lower ends of ablation range were obtained from the deadzone plot (Figure 2B). For a particular additive step with  $P_{add}$ of 150 mW and  $t_{add}$  of 20 s, the dead-zone thickness was measured as 30  $\mu$ m, while for a particular subtractive step with  $P_{sub}$ of 1200 mW and  $\nu_{sub}$  of 100  $\mu m~s^{-1},$  the upper end of the range was measured to be 160 µm, giving an "ablation z-range" of  $\approx$ 35–160 µm. In a similar fashion, ablation z-ranges were calculated for a range of laser powers and scanning speeds, and these parameters were used to automate the HLP platform.

Next, the amount of material removal during the subtractive step of HLP was characterized in term of ablation linewidth. To do so, ablation laser powers ( $P_{sub}$ ) and scanning speeds ( $v_{sub}$ ) were varied and the widths of the ablated lines were measured. First, hydrogel slabs were cross-linked with  $P_{add}$  of 150 mW and  $t_{add}$  of 20 s. Second, laser ablation was carried out at a depth of 100 µm from the Teflon surface, and width of the ablated lines was recorded by averaging five readings. As shown in Figure 2D, results show that the ablated linewidth decreases from 8 to 3 µm with an increase in scanning speeds ( $v_{sub}$ ) and a decrease in ablation laser powers ( $P_{sub}$ ). These data were used to determine the ablation laser power and scanning speed for achieving desired ablation linewidth.

Our result suggests that the minimum feature size of the ablated line is around 3 µm, and this value is higher compared to resolution based on Abbe's resolution criteria. Considering Abbe's criteria, resolution is given by  $d = \lambda/(2 \times NA)$ , where  $\lambda$  is wavelength of light and NA is numerical aperture. For two-photon absorption, the resolution improves roughly by a factor of  $\sqrt{2}$ .<sup>[7]</sup> For a laser wavelength of 800 nm and numerical aperture of 0.55, the theoretical resolution of our optical setup should be ~514 nm. However, the mechanism of fs ablation of the hydrogel is dictated by the shockwave generation and bubble formation that extend beyond the laser focus spot and increases the feature size of ablated structure. Moreover, we only report the linewidth of fully ablated lines, and do not take into account partial ablation or incomplete ablation events.

Next HLP was used to print complex 3D structures with internal microfeatures. To test the achievable design complexity of HLP, a Mayan pyramid, with a hollow cube frame embedded within a 3D pyramid structure, was fabricated using 90% PEGDA, 1% LAP prepolymer solution. Briefly, a 3D model of a Mayan pyramid is sliced to generate digital masks for the

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Figure 2. A) Schematic showing key parameters in HLP. Ablation z-range and dead zone are two key parameters, which are highlighted in the figure by curly bracket. Please note that the additive and subtractive processes are sequential processes, although they are depicted in the same figure for simplicity. B) Plot of dead-zone thickness as the function of exposure time and laser power. As for an instance, PEGDA slab cross-linked using crosslinking laser power of ( $P_{add}$ ) of 150 mW and exposure time ( $t_{add}$ ) of 20 s (marked by black arrow) results in dead zone of 30  $\mu$ m. C) Schematic cartoon showing lines ablated at different penetration depths and plot of ablated linewidth as a function of laser penetration depths and laser powers. Results shows a decreasing linewidth trend with increasing laser penetration depth. Red marks in the plot represent the upper limit of ablation z-range. D) Plot of ablated linewidth as a function of scanning speeds and laser powers. Plot shows a decreasing linewidth trend with decreasing laser dose. For all of the studies in this figure, 90% PEGDA and 1% LAP was used as material for fabrication.



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**Figure 3.** A) Diagram showing slicing of an stl model of Mayan pyramid to create a stack of digital masks. B) Key steps in the HLP process to print a Mayan pyramid with embedded cube frame. Iteration of additive and subtractive steps are involved to print a complete "cube inside pyramid" structure C) Side, top and isometric views of "cube inside pyramid" structure in 90% PEGDA, 1% LAP and the image is recorded by an HIROX digital optical microscope. For additive step laser power of 200 mW and laser wavelength of 400 nm was used, while laser power of 1200 mW, scanning speed of 100  $\mu$ m s<sup>-1</sup>, and laser wavelength of 800 nm was used for subtractive steps. The height of the pyramid is 6 mm. The cube is 600  $\mu$ m<sup>3</sup>, embedded 2 mm from the bottom of the pyramid (red arrow points to the embedded cube).

DMD using a custom written algorithm, and additive-subtractive sequential processes were used to print the structure in an automated manner (Figure 3A,B). For the additive steps, laser wavelength ( $\lambda_{add}$ ) of 400 nm and cross-linking laser power ( $P_{add}$ ) of 200 mW was used to selectively cross-link PEGDA layers in a continuous fashion onto a methacrylated glass coverslip. For subtractive steps, a laser wavelength ( $\lambda_{sub}$ ) of 800 nm, an ablation laser power ( $P_{sub}$ ) of 1200 mW, and a scanning speed ( $v_{sub}$ ) of 100 µm s<sup>-1</sup> was used to ablate a hollow cube frame within the cross-linked layers. (Figure 3B). The additive and subtractive process was iterated in an automated fashion to fabricate a Mayan pyramid (6 mm tall) with an embedded cube frame (600  $\mu$ m<sup>3</sup>). The location of the cube is 2 mm inside the pyramid from the base of the pyramid (Figure 3C). A total fabrication time of 66 min was dominated by the time required for subtractive steps (62 min) as compared to all the additive steps combined (3.6 min) and the time required to transition between steps (0.4 min). This work demonstrates HLP's ability to remove material at any depth within 3D structures. As compared to current laser-based fabrication methods where laser penetration depth is substantially influenced by the optical properties of the prepolymer solution, HLP is less dependent on the optical properties of the prepolymer solution due to the sequential additive and subtractive modes of fabrication that enables the ablation of materials from any depth within a 3D structure.

To further demonstrate HLP's printing capabilities, openwell chips were printed using 10% PEGDA 1% LAP (**Figure 4**). Multilayer 1.5 mm thick chips that consist of two open rectangular wells (1.25 mm × 2.5 mm) connected by either inplane microchannels (6  $\mu$ m in diameter) (Figure 4A) or out-ofplane microchannel (8  $\mu$ m) were printed (Figure 4B). For both these structures, the total printing time was 4.5 min. For chips with embedded in-plane microchannels, DMD-modulated laser beam was used to print rectangular wells using a  $\lambda_{add}$  of 400 nm,  $P_{add}$  of 150 mW, and  $t_{add}$  of 20 s followed by ablation of microchannels in straight, zig–zag, and square-wave configurations using a  $P_{sub}$  of 1200 mW and  $v_{sub}$  of 100 µm s<sup>-1</sup>. Repeated scans (2 times) are necessary to ensure that channels are not blocked with any debris. Rhodamine B dye, pipetted in one of the wells, was observed to perfuse within the channels under static hydrostatic pressure as shown in Figure 4A. Chips with out-of-plane 3D microchannels were fabricated using  $P_{add}$  of 150 mW,  $t_{add}$  of 20 s in the additive mode, and  $P_{sub}$  of 1300 mW and  $v_{sub}$  of 50 µm s<sup>-1</sup> in the subtractive mode. Sequential additive and subtractive modes in HLP allow the ablation of 3D channel that span across multiple cross-linked layers (Figure 4B).

To demonstrate the utility of HLP for potential bioscience applications, 4-well PEGDA chips with embedded microchannels were printed (Figure 5). To assess the utility of such chips in studying cell-cell communication, we choose model osteocyte cell line. In the bone tissue, osteocytes reside within isolated cavities (lacunae) and they communicate with their neighboring cells by forming dendritic processes within hollow microchannels called canaliculi. To mimic this microenvironment, PEGDA chips, 200 µm thick, with 40 microchannels (diameter 5  $\mu$ m) were ablated within walls (width =  $100 \ \mu m$ ) that separate adjacent wells. These chips were printed using a  $P_{add}$  of 150 mW,  $t_{add}$  of 20 s,  $P_{sub}$ of 1200 mW, and  $\nu_{sub}$  of 200  $\mu m~s^{-1}.$  For the fabrication of these chips, a total printing time of 3 min was required. Mice MLO-Y4 osteocytes, seeded within the chips, remain isolated in their respective wells; however, they extend cell processes through the microchannels to establish direct physical contact with cells seeded in the adjacent well. This is confirmed by the presence of the nucleus on the either side of the wall, while only cell processes labeled by f-actin (green) is present within the channel (Figure 5A).







**Figure 4.** Printing user-defined two-well PEGDA chips with interconnecting in-plane and out-of-plane microchannels. A) In-plane channels: side and top views of channels with zig-zag and square-wave configurations are depicted. Perfusion of Rhodamine B through zig-zag channels is demonstrated. B) Out-of-plane channels: Side, isometric and top views of 3D channels spanning multiple cross-linked layers are depicted. White dotted box highlights the zig-zag ablated channels. For all of the studies in this figure, 10% PEGDA and 1% LAP was used for fabricating the structures.

To potentially extend the utility of these chips to cell migration studies, the chip design was modified by increasing the wall thickness to 300  $\mu$ m. A  $P_{sub}$  of 1400 mW and  $\nu_{sub}$  of 50  $\mu$ m s<sup>-1</sup> was used to ablate channels of diameter 7  $\mu$ m, a size that would facilitate cell migration within the channel (Figure 5B). Model human Saos-2 osteosarcoma cell line, chosen for this work, is able to migrate within the channels, as indicated by the presence of both f-actin (green) and nucleus (blue) within the interconnecting channels on day 4. For these chips, a total printing time of 11 min was required to account for the increased wall



**Figure 5.** A) Brightfield image of a 4-well PEGDA chips with interwell microchannels is depicted. Fluorescent images showing actin (green) and nucleus (blue). MLO-Y4 cells seeded in neighboring wells communicate with each other by extending cell processes within the ablated channels. White arrows point the communication channels with a diameter of 5  $\mu$ m. B) Brightfield image of a 4-well PEGDA chips with interwell microchannels is depicted. Seeded osteosarcoma Saos-2 cells migrate within the ablated channels. White arrows show the migration channels with a diameter of 7  $\mu$ m. For all of the studies in this figure, 10% PEGDA and 1% LAP was used for fabricating the open-well chips.



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**Figure 6.** HLP's capability of additive–additive multiscale printing. A) Schematic depicting sequential additive steps of DMD-based printing (CLIP) and multiphoton polymerization (MPP). Three woodpile structures were printed in different depth using MPP. B) Corresponding DMD masks and wood pile structures. C) HIROX digital optical microscopy image depicting three woodpile structures fabricated at different z-planes and xy spatial locations. Three different woodpile structure marked by 1, 2, and 3 correspond to the zoomed images to the left. Structure was printed using 90% PEGDA and 1% LAP with laser power of 200 mW and exposure time of 5 s in additive CLIP step. While high resolution MPP fabrication was performed using laser power of 400 mW and scanning speed of 100  $\mu$ m s<sup>-1</sup>.

thickness and channel sizes. Based on Figure 5A,B, we can say that the resulting channels are open and are not clogged by debris. The process is not toxic to cells as seen in the figures, where seeded cells reliably migrate and form cell–cell connections within the microchannels. To demonstrate that HLP can be extended to other photosensitive hydrogels, 4-well chips using naturally-derived gelatin methacrylate (GelMA) hydrogel were printed using a  $P_{\rm add}$  of 200 mW,  $t_{\rm add}$  of 25 s,  $P_{\rm sub}$  of 100 mW, and  $\nu_{\rm sub}$  of 100  $\mu$ m s<sup>-1</sup>. (Figure S2, Supporting Information )

### 4. Additive/Additive Mode of Hybrid Laser Printer

HLP in additive DMD-based printing (CLIP) and additive MPP was used to achieve on-demand fabrication of multiscale

3D structures with superior design flexibility. To demonstrate this capability, we choose to use model prepolymer solution (90% PEGDA, 1% LAP) to print a multitier design that consists of three log-pile structures printed on different Z-heights and XY locations. Figure 6A illustrates the sequence of steps, while Figure 6B shows the series of masks for additive CLIP and laser paths during the additive MPP steps. In the first step, a 200 µm thick layer was additively printed using CLIP (Mask 1). In the second step, MPP was used to print the first woodpile structure. This is followed by Step 3, where another 200 µm thick layer was printed using Mask 2 (CLIP). Similarly, sequential use of MPP and CLIP was used to print the rest of the structure (Steps 3-6). To clearly visualize the embedded 3D logpile structures in different z-planes without mechanical sectioning, a rhombus shaped blank structure was added to the DMD masks. Figure 6C depicts a representative HIROX digital



microscopy image of HLP-printed 3D multiscale structure. In this study, additive CLIP steps used a laser power of 200 mW and exposure time of 5 s, while additive MPP steps used a laser power of 400 mW and scanning speed of 100  $\mu$ m s<sup>-1</sup> using an objective lens (Zeiss, 20X) with numerical aperture of 0.25. The logpile structures consists of 10 layers of logpile structure (logsize = 2  $\mu$ m; log-spacing = 20  $\mu$ m). Each logpile structure is  $\approx$ 80  $\mu$ m thick and each structure is printed at different z-levels/heights. (Figure S3, Supporting Information ) The total fabrication time of 45 min is mostly dictated by the MPP steps. This work demonstrates that the HLP is capable of fabricating 3D multiscale structure using multistep and automated additive–additive approach.

### 5. Multimaterial Printing Mode of Hybrid Laser Printer

To demonstrate the HLP's capability of printing multimaterial 3D structures, we built a new fluid chamber with necessary tube connections to enable efficient switching of different prepolymer solutions (Figure 7). The fluid chamber was designed using Autodesk Inventor and machined using a vertical mill. The sample holder consists of the five 3 mm diameter inlets, printing region (also known as fabrication window) of 1 cm in diameter, and five 3 mm diameter outlets. Three inlets were used to pump different hydrogel prepolymer solutions, while the fourth and the fifth inlets were designed to pump in washing solution and/or nitrogen. Similarly, three of outlets were designed to pump-out the prepolymer solution to the respective syringes so that the solutions can be reused. The other two outlets were used to drain the DI water during washing steps; this step is necessary to avoid mixing of different prepolymer solutions during printing. A circular hole (diameter 10 mm) was drilled in the center of the sample holder and a thin Teflon film was glued. This area is named as fabrication window where the exposure of the hydrogel prepolymer solution takes place. The floor of the fluid chamber was sloped so that the prepolymer solutions can be easily drained during the washing steps. Before introducing new prepolymer solution, compressed nitrogen was used to ensure the complete removal of DI water. While printing GelMA structures, the heater (shown in Figure S1 in the Supporting Information) was maintained at 40 °C. This setup allowed for repeatable printing of multimaterials structures with no mixing of the prepolymer solutions.

HLP in DMD-based additive mode (CLIP) was used to demonstrate the multimaterial printing capability in both the z(Mayan pyramid, Figure 8A) and the xy (yin-yang, Figure 8B) directions. Figure 8A shows digital masks and image of a Mayan pyramid printed using three solutions composed of 90% PEGDA hydrogel, 1% LAP photoinitiator and food color dye of different colors (blue, green, and red). This structure was printed using the cross-linking laser power  $(P_{add})$  of 200 mW, a printing speed in the vertical direction of 0.02 mm s<sup>-1</sup>, and an exposure time per layer ( $t_{add}$ ) of 3 s. Similarly, yin–yang structure was printed using a laser power of  $(P_{add})$  200 mW and exposure time  $(t_{add})$  of 5 s for each step (Figure 8B). Next, additive DMDbased mode (CLIP) was combined with additive MPP to print nested logpile multiscale 3D structures using three different polymer compositions (Figure 8C). First, CLIP was used to print the outer thick pink wall using 20% GELMA, 0.25% LAP doped with Rhodamine B dye. Second, CLIP was used to print internal walls (that separate the wells) using 50% PEGDA, 1% LAP. For both these structures, a cross-linking laser power  $(P_{add})$  of 200 mW and exposure time  $(t_{add})$  of 5 s was used. Third, MPP was used to print high resolution logpile structure using a laser power of 400 mW and scanning speed of 100 µm s<sup>-1</sup>. The laser beam was focused using 20X objective lens (Zeiss, NA = 0.25)



Figure 7. A) Syringe pump system for multiscale, multimaterial fabrication. Three different inlets and three outlets were used for exchange of printing material. Nitrogen/water inlets and two water outlets were meant for washing and drying steps. B) Side view of the fluid chamber, which depicts five inlets/five outlets and fabrication area. Cross-section view (at marked green dotted line) shows the sloped floor design that facilitates easy exchange of prepolymer solutions during printing.





**Figure 8.** A) Schematic depicting digital mask and multicolored Mayan pyramid fabricated using multimaterial additive printing of three colored 90% PEGDA, 1% LAP prepolymer solutions. B) Yin and yang structure printed using four different colored 90% PEGDA, 1% LAP. C) Multiscale printing of multimaterial structure using two commonly used hydrogel (GeIMA and PEGDA). The outer thick wall colored in pink is printed with 15% GELMA, 0.5% LAP using DMD-based fabrication. Similarly, the DMD-based approach is used to print intersecting lines using 50% PEGDA, 1% LAP. Further logpile structure shown inside the black square box is printed using 90% PEGDA, 0.5% LAP (colored in green) and 50% PEGDA, 0.5% LAP (colored in red). Insets shows the zoomed version of ablated lines using MPA.

to obtain a minimum feature size of 2 µm. Next, multimaterial 3D printing capability was demonstrated by combining additive CLIP and subtractive MPA modes. Additive CLIP was used to print structures using two hydrogel compositions (90% PEGDA, 0.5% LAP with green food dye, 50% PEGDA, 0.5% LAP, with red food dye) with a laser power ( $P_{add}$ ) of 200 mW and exposure time  $(t_{add})$  of 5 s. (Figure 8D) Laser processing conditions to achieve a specific ablation size within both materials (Figure S4, Supporting Information) were used to ablate an array of channels in both regions using MPA. For this step, laser power was kept constant ( $P_{sub} = 1500 \text{ mW}$ ) and the scanning speed was switched between 50 to 150  $\mu m~s^{-1}.$  The plot shown in Figure S4 in the Supporting Information can be explicitly used to ablate desired linewidth structures in these material. For instance, to print a uniform channel of (≈7.6 µm) throughout the both slabs, the scanning speed is required to maintain at 130  $\mu$ m s<sup>-1</sup> inside 90% PEGDA slab and 50  $\mu$ m s<sup>-1</sup> inside the 50% PEGDA slab.

#### 6. Discussion

#### 6.1. Comparison of HLP with Existing Fabrication Techniques

Contrary to materials found in nature that possess 3D structural hierarchy and material heterogeneity, man-made materials remain relatively simple. Current manufacturing technologies are limited by a trade-off between the use of multiple materials, overall size range, dimensionality, throughput and resolution. (Table S1, Supporting Information) For instance, subtractive methods based on lithography (photo-, soft-, nanoimprintlithography) exhibit excellent feature resolution, however these methods typically generate planar devices or they required complicated multiple bonding and stacking steps to fabricate devices with even simple 3D designs.<sup>[8,13]</sup> On the other hand, additive manufacturing methods such as FDM, MJM, and DLP offer 3D design flexibility, however achieving microscale resolution with these methods remain challenging.<sup>[2-4]</sup> Among the various fabrication methods at our disposal, ultrafast lasers, with their unique property of nonlinear multiphoton absorption, have revolutionized the processing of materials at micrometer scale using MPP and MPA.<sup>[14,15]</sup> In this work, we have designed and built a single versatile manufacturing platform coined as HLP. HLP, by combining additive CLIP with additive MPP and subtractive MPA processes, enables quick printing of centimeter-sized hydrogel chips with embedded hollow or solid microfeatures; this would otherwise require, multiple planar fabrication followed by the complex alignment of multiple components using conventional lithography. Integration of subtractive MPA with CLIP also ensures reliable removal of material in defined locations. This is a clear advantage as compared to current 3D printing methods where removal of support or sacrificial material from micro-channels/features remains challenging. Using several proof-of-concept studies, we demonstrate HLP's ability to shape soft hydrated and difficult-to-process hydrogel materials into complex multiscale structures that are either highly challenging, or time consuming to fabricate, or cannot be fabricated using current methods.<sup>[16]</sup>



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#### 6.2. Comparison of HLP with Existing Laser-Based Hybrid Methods

Only handful researchers have developed hybrid additivesubtractive methods using lasers due to the material incompatibilities and/or processing requirements.[10,11] One approach, that has been widely used, has combined ultrafast lasers in additive (MPP) and subtractive (MPA) modes to fabricate complex 3D structures using epoxy-based photoresists. However, time-consuming serial nature of the both MPP and MPA has limited the scalability of these methods and therefore their utility in the field for making centimeter sized devices.<sup>[8,12]</sup> In comparison, HLP combines additive CLIP with MPP/MPA processes to enable printing of centimeter-sized hydrogel chips with embedded microfeatures within minutes. Another laser hybrid method utilizes specialized materials such as Foturan photosensitive glass to make hollow microfeatures. This method involves two steps. In step 1, laser irradiation is used to modify the material properties to allow facile removal of materials using chemical etching. In step 2, MPP is used to cross-link complex 3D structure within the chemically etched channels. Since processing requirements for the etching and MPP steps are distinct, this approach cannot be automated into a multistep multilayer process. Additionally, the use of harsh chemical and processes (etching and high temperature treatment) makes this process incompatible with hydrogel materials. In comparison, HLP technique can print 3D structures with embedded features at a resolution of few micrometers using a multistep multilayer automated process without the use of any harsh processing steps, a key materials criteria when working with soft hydrogel materials. Although we have utilized model synthetic PEGDA and naturally-derived GelMA hydrogels in this work, we anticipate that HLP can be extended to other photosensitive materials.

# 6.3. HLP Can Fabricate Hollow Microfeatures at Any Depth within a 3D Structure

Laser-based methods such as MPA depend heavily on the optical properties (transparency, absorption, scattering) of the material.<sup>[15-17]</sup> Low laser penetration depths limit the processing range of subtractive ablation within the materials. In contrast, HLP technology allows the fabrication of hollow microfeatures at any depth within a complex user-defined 3D microstructures. This unique feature is demonstrated by the printing of i) an embedded hollow cube frame deep within a Mayan pyramid 3D structure (Figure 3C), ii) an embedded out-of-plane microchannel within a two-well PEGDA chip (Figure 4B), and iii) 3D logpile structures at different depth/heights (Figure 6). The sequential additive-subtractive modes make this technique more-or-less independent of stringent processing depth limits. HLP can be potentially used to fabricate 3D structures using less transparent materials as well, thus decreasing the dependence of laser penetration depths on the optical properties of the materials.

# 6.4. HLP as Compared to Current Light-Based Multiscale Fabrication Methods

Laser-based methods that combine two separate additive processing steps or methods have been used to fabricate multiscale 3D structures.<sup>[18–21]</sup> For instance, SLA was used to print multiscale surface features with a resolution of 37  $\mu$ m by adaptively switching the laser spot and slice layer thicknesses.<sup>[19]</sup> Shaped laser beams with adaptive layer thicknesses were used to print 3D multiscale structures with a resolution of 30  $\mu$ m, although hollow microscale features were not reported.<sup>[20]</sup> Large area multiscale printing was demonstrated by synchronizing linear scanner with high speed capability of galvanoscanners.<sup>[21]</sup> As compared to the methods described above, HLP can print 3D multiscale structures with a smallest feature size of 3  $\mu$ m. Figure 6 highlights the quick fabrication of 3D multiscale structures with superior design flexibility.

# 6.5. HLP as Compared to Current Light-Based Multimaterial Fabrication Methods

Few light-based methods have been adopted for multimaterial printing as explained below.<sup>[22–27]</sup> SLA- and DMD-based optical lithography have been used to print multiple low viscosity resins,<sup>[23]</sup> and hydrogel-based materials.<sup>[24]</sup> To improve the fabrication speed, DMD-SLA was recently combined with a microfluidic device<sup>[25]</sup> and an air-jet<sup>[26]</sup> to achieve automated and quick material exchanges. Recently, a commercial direct laser writing system was combined with a microfluidic chamber to enable 3D multimaterial printing.<sup>[27]</sup> As compared to the current methods, HLP enables multimaterial printing in additive–additive (CLIP-CLIP), additive–additive (CLIP-MPP), and additive–subtractive (CLIP-MPA) modes (Figure 8). By combining CLIP with MPP and MPA, HLP achieves a suitable trade-off between the use of multimaterials, overall throughput, size-range and feature resolution, factors critically important in manufacturing 3D multiscale structures.

The HLP technology can be further improved to enable new capabilities. For instance, the maximum size of printable 3D structure can be increased by simply modifying the polymer chamber, and by adding new inlet channels can be used to increase the number of materials. Additionally, the speed of multimaterial HLP can be further improved by adopting a continuous stop-flow lithography method and automation of fluid exchange and printing processes.<sup>[28]</sup> Furthermore, future studies can be made to better the understand the ablation mechanism, which is not currently known. Based on known literature, the formation of shockwaves and cavitation, likely led to a disruptive breakdown of the hydrogel matrix and resulted in the generation of voids during the subtractive ablation mode of HLP, however systematic studies are required.<sup>[11]</sup> In the future, it is also advisable to study the chemical composition of the ablated regions, which is at this point difficult due to their microscale size of the ablation voids and mechanically weak nature of hydrogel materials.

In essence, we anticipate that HLP has the potential to revolutionize the ability to make 3D multiscale multimaterial structures, specifically those structures that consist of internal or embedded hollow features that cannot be made using current technologies.

### 7. Conclusion

Due to material incompatibilities and significant differences in laser processing requirements of additive and subtractive

processes, only few research groups have investigated hybrid fabrication approaches. In this work, we have designed and developed a new HLP technology that seamlessly combines additive cross-linking and subtractive ablation modes of femtosecond laser to achieve the printing of 3D multiscale multimaterial structures using difficult-to-process hydrogel materials. Quick fabrication of multiscale structures with embedded hollow microfeatures demonstrates superior design flexibility of HLP as compared to conventional lithography methods, with a resolution close to that achieved by lithography. The ability to print multimaterials in additive-additive and additive-subtractive modes demonstrates the fabrication versatility of HLP. This capability can be potentially used to print 3D multimaterial hydrogel-based structures for a variety of applications in biomedical sciences, microfluidics, soft robotics, optics, photonics, and other application areas.

### 8. Experimental Section

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Optical Setup for HLP: The schematic of the custom-built hybrid laser printing setup is shown in Figure 1. Both additive and subtractive modes of HLP utilize a femtosecond (fs) laser source (Coherent, Chameleon-Ultra Ultrafast Ti:Sapphire) capable of producing 150 fs wide pulses at a repetition rate of 80 MHz, with tunable fundamental wavelength ranging from 690-1040 nm. In the additive mode of HLP (shown by blue arrow in Figure 1), fs-laser beam was passed through a SHG (Autotracker, Coherent) to obtain an ultraviolet/near visible wavelength. A shutter (SH05, Thorlabs Inc) was placed after SHG generator and 2f-transfer lens (f = 40 and 200 mm) assembly, which collimated and expanded the laser beam. A pin hole of 25  $\mu$ m was used to spatially clean the laser beam. The Gaussian intensity distribution of the laser beam was changed to hat-top intensity distribution using an engineered diffuser (RPC photonics Inc.) in a rotating mount and the beam was spatially modulated via a DMD (0.95" 1080p UV DMD, DLi Inc). DMD is a spatial light modulator consisting of an array of micromirrors. User can selectively switch mirrors into either an ON state or an OFF state to modulate laser beam in user-defined light-patterns using a computer generated digital mask. A DMD-modulated fs-laser beam was projected to the sample with the infinity corrected projection optics. Laser beam modulated by the DMD can be used to cross-link photosensitive hydrogels in both continuous and layer-by-layer manner, with a feature size ranging from  $\approx$ 30  $\mu$ m to 1 cm. A custom written MATLAB code was used to slice a 3D model of the structure and to obtain a series of DMD mask.

The subtractive mode of HLP utilizes the fs direct laser writing to fabricate structure using multiphoton ablation (MPA) process. However, this mode can also be used as multiphoton polymerization (MPP) mode simply by adjusting the laser intensity. The fs laser beam tuned at 800 nm was passed through an assembly of a half-wave plate and a polarizing beam splitter, which was used to adjust the intensity of the incident laser beam. The laser beam was spatially cleaned and collimated using a 2f-transfer lens (f = 40 and 200 mm) assembly and a 25  $\mu$ m pinhole. The beam was directed toward the dichroic filters (Semrock, FF395/495/610-Di01-25  $\times$  36) which reflects the laser beam toward objective lens (Carl Zeiss, 50X, 0.55 NA). The objective lens focused the laser beam into the previously cross-linked hydrogel layer. Material from predefined region was ablated when the laser intensity exceeded the ablation threshold of the cross-linked hydrogel layer. The in situ fabrication process can be monitored in real-time using a microscopy arm incorporated in the setup. A custom-written LabVIEW program was used to synchronize the additive and subtractive processes, including switching the DMD masks, turning the shutter ON and OFF, and moving of XYZ stages. It is worth noting, that for all the experiments, laser power was measured at the back aperture of the objective lens, however for the additive cross-linking, power  $(P_{add})$  was measured just

before the DMD. The coverslips used in this work were methacrylated to ensure adhesion of cross-linked structure.

*Measurement of Linewidth*: The linewidth of the structure was imaged by Zeiss microscopy and measured via ImageJ software. Line profiles of the structures were plotted and full width at half maxima (FWHM) was recorded to obtain the linewidth. The linewidth of the structures was averaged from five line profiles.

Surface Modification of Glass Substrate: Glass coverslips (18 mm x 18 mm, No.1; Globe Scientific) were surface modified with methacrylated function group to ensure adhesion of cross-linked structure to the glass during HLP printing. Briefly, glass coverslips were treated in piranha solution (sulfuric acid and hydrogen peroxide, 7:3 ratio) for 20 min, washed with MilliPore water and 100% ethanol (Fisher Scientific, Pittsburgh, PA), blow dried using compressed air, and soaked in a bath containing  $85 \times 10^{-3}$  M 3-(trimethoxysilyl) propyl methacrylate (Fluka, St. Louis, MO) in ethanol with acetic acid (pH 4.5) for 12 h at room temperature, and subsequently washed in 100% ethanol and air dried.

IAP phenyl-2,4,6-Photoinitiator Synthesis: Lithium trimethylbenzoylphosphinate (LAP) was synthesized in a two-step process based on an established method.<sup>[29]</sup> At room temperature and under argon, 2,4,6-trimethylbenzoyl chloride (4.5 g, 25 mmol) was added dropwise to continuously stirred dimethyl phenylphosphonite (4.2 g, 25 mmol). This solution was stirred for 24 h before adding an excess of lithium bromide (2.4 g, 28 mmol) in 50 mL of 2-butanone to the reaction mixture at 50 °C, to obtain a solid precipitate after 10 min. The mixture was then cooled to room temperature, allowed to rest overnight and then filtered. The filtrate was washed with 2-butanone (3  $\times$  25 mL) to remove unreacted lithium bromide and dried under vacuum to give LAP (6.2 g, 22 mmol, 88% yield) as a white solid.

Preparation of PEGDA and GelMA Prepolymer Solutions: Different concentrations of Poly (ethylene glycol) diacrylate (PEGDA, Mw = 700, Sigma-Aldrich) prepolymer solutions were prepared for this project. First, LAP (0.4 g) was added to 4 mL of Millipore water and mixed thoroughly using Vortex mixer (Vortex-Genie 2 from Fisher Scientific). Second, 36 mL of 100% PEGDA hydrogel was added to the 4 mL of LAP solution to prepare 90% PEGDA solution and further mixed in Vortex mixer, and incubated at 37 °C to remove the bubbles. Similarly, 10% and 50% PEGDA prepolymer solutions were also prepared.

Gelatin Methacrylate (GelMA) macromer was synthesized as described previously.<sup>[30]</sup> Briefly, 200 mL Dulbecco's phosphate buffered saline (DPBS, Gibco) was heated to 40 °C, and was used to dissolve 10 g of porcine skin gelatin (Sigma Aldrich, St. Louis, MO). Further, methacrylic anhydride was subsequently added to the solution and stirred for 3 h. The unreacted groups from the solution was removed by dialyzing the mixture against distilled water for a week at 40 °C. Next, the dialyzed GelMA was lyophilized in a freeze dryer (Labconco, Kansas City, MO) for another week. A stock solution was repared by mixing 0.75 g freeze dried GelMA with 10 mL of deionized water at 40 °C and 0.01875 g of LAP photoinitiator to obtain a 10% (w/v) GelMA with 0.25% LAP (w/v).

*Cell Culture*: All the cells used in the experiment were cultured under standard cell culture conditions (5% CO<sub>2</sub>; 37 °C). MLO-Y4 osteocytes were maintained in  $\alpha$ -Modified Eagle Medium ( $\alpha$ -MEM, Gibco) supplemented with 2.5% heat inactivated FBS, 2.5% calf serum (CS), 1% P/S and 1% Glutamax on rat tail type I collagen-coated flasks, and passage 9–15 were used. Saos-2 human osteosarcoma cells were maintained in DMEM supplemented with 10% FBS, 1% P/S, and 1% Glutamax, and were passaged when 70–80% confluence was reached.

*Cell Culture within PEGDA Chips*: Multiwells PEGDA chips were designed and printed using HLP. The PEGDA multiwell chips were coated with rat tail type I collagen at 37 °C overnight and then flushed with DPBS before seeding either MLO-Y4 osteocytes or Saos-2 osteosarcoma cells at a final concentration of  $1 \text{ M mL}^{-1}$ . Specifically, a 2 µL cell solution was added to the chips, and placed in the incubator (37 °C) for 20 min to allow for sufficient attachment of the cells, before adding medium to the entire chip and culturing them under standard culture conditions. Live/Dead assay kit (Invitrogen) (Calcein AM, Ethidium homodimer)



was used to quantify the viability of cells after 3 days of culture. Briefly, a solution of 0.5  $\mu$ L mL<sup>-1</sup> calcein AM and 2  $\mu$ L mL<sup>-1</sup> ethidium homodimer in BME was pipetted into PEGDA biochips, incubated at 37 °C for 45 min before capturing images using an inverted microscope (Nikon Eclipse). To determine cellular morphology, cells cultured within chips were fixed with 4% formaldehyde (Invitrogen, Carlsbad, CA) for 30 min, soaked in 2% Triton X-100 in DPBS for 30 min, and subsequently stained with phalloidin (Alexa-Fluor 568, Invitrogen) (1/100 dilution in DPBS) (45 min at RT) and with 1.25  $\mu$ g mL<sup>-1</sup> DAPI (Life Technologies) (5 min at RT) to visualize f-actin and cell nuclei respectively.

Digital Optical Microscope Imaging: The fabricated structures were imaged and characterized using a digital optical microscope (HIROX, KH-8700). The MXG-2016Z /MX-2016Z 20–160× objective lens was used to image the fabricated structures, which provided a resolution of 1.35  $\mu$ m.

### **Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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### **Conflict of Interest**

The authors declare no conflict of interest.

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