MULTI-SCALE 3D PRINTING OF BIOINSPIRED STRUCTURES WITH FUNCTIONAL SURFACES

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ABSTRACT
Nature provides inspirations of creative multi-functional materials and structures. However, biological systems are usually composed of materials and structures in multi-scales with high geometry complexity, which brings challenges to bioinspired design and fabrication. Additive manufacturing (AM) presents a potential solution due to its capability of creating three-dimensional (3D) objects with freeform surface and multi-materials. The aim of this research is to investigate the fabrication of multi-scale biomimetic structures using the integrated photo-polymerization based 3D printing processes. We firstly introduced the macro- and meso-scale mask image projection based stereolithography (MIP-SL). We then present the micro-scale immersive surface accumulation (ISA) printing. Based on them, a multi-scale 3D printing method by integrating the ISA and MIP-SLA processes is presented. To achieve high-resolution fabrication, the process planning, the material curing performance, and the printing parameters are discussed. Two test cases are presented to demonstrate its fabrication capability. The experimental results verify the accuracy and efficiency of the developed multi-scale 3D printing method on the fabrication of bioinspired structures with functional surfaces.

NOMENCLATURE
\( C_d \): The cure depth of photocurable material
\( D_p \): The penetration depth of exposure light
\( E_c \): The critical exposure of photocurable material
\( E_{max} \): The energy of light exposure
\( L \): The distance between the printed object and the light guide tool

INTRODUCTION
Throughout a long term of evolutionary process, nature creates biological systems in an efficient and elegant way with impressive multi-functional structures and materials [1]. Due to unique three-dimensional (3D) structures encompassing multi-materials from different material categories and a myriad of scales from nanoscale to macroscale, the biological systems have shown impressive and promising performances in diverse frontiers such as optics, mechanics, acoustics, electricity, thermionics, magnetics and medical science as shown in Fig.1 [1-6]. Such smart materials and multi-scale structures in nature provide inspirations for innovation in engineering system development. For example, inspired by mosquito compound eyes, researchers designed and fabricated artificial micro lens array on complementary metal–oxide–semiconductor (CMOS) image sensor for autonomous vision, enabling distortion free imaging with wide field of view at large range spectral region [1]. Besides animals, plants also possess inspiring functional mechanisms. For example, some plants show attractive phenomena due to the hierarchical structures with longitudinal nanostructures and asymmetric microstructures, such as superhydrophobicity of rose petal, self-cleaning of lotus leaf, and drag reduction and oil cleaning of salvinia paradoxa [4].

Along with the research on nature-inspired bionic structures and systems, understanding and replicating the mechanism of multi-scale hierarchical structures in nature have become increasingly important. This also presents critical demand for high-resolution multiscale manufacturing techniques that can fabricate such multi-scale hierarchical structures. However, most of the traditional fabrication techniques...
Several multi-scale additive manufacturing (AM) processes have been developed before to reproduce bioinspired functional structures. For example, the dimension of a 3D printed object can be controlled by the nozzle size of the extruders in the fused deposition modelling (FDM). Hence multi-scale structures can be fabricated by using different nozzle sizes of the printing heads [9,10]. Similarly, in the laser-based stereolithography (SL) process, 3D bioinspired structures with microscale and nanoscale features can be fabricated by the high-speed voxel modulation laser scanning (HVLS), which can select appropriate laser voxel dimension based on the fabrication scale requirements [11]. Furthermore, multiple structural colors inspired by the nature organism were fabricated using the inkjet printing process and composite materials with photonic crystal [12]. Instead of only using a single 3D printing process, multi-scale structures can also be fabricated by integrating different 3D printing processes. Previous works on combining multiple 3D printing processes to fabricate objects with features ranging from macroscale to nanoscale based on various materials were reported [13-15]. Other manufacturing processes can also be integrated with AM technology to fabricate multi-scale bioinspired structures. For example, to mimic structured color of butterfly wings, multi-scale large-area negative index metamaterials were fabricated using the transfer printing method consisting of multiple fabrication processes including laser-based stereolithography, material deposition, and nanoscale transfer printing [16]. Overall, 3D printing multi-scale biomimetic structures by integrating multiple manufacturing processes would be beneficial if the fabrication capability of each manufacturing process can be fully utilized to achieve their desired functional performance.

The stereolithography apparatus (SLA) process is an additive manufacturing process with good properties such as high precision and fast fabrication speed. The SLA technology has been widely used in various applications. However, current SLA processes including both laser-based and mask-projection-based can only achieve a single scale size with the ratio of fabrication extent to fabrication resolution at around 500. However, multi-scale hierarchical structures in nature contain features from macroscale to nanoscale, where the ratio of the size span to the smallest geometry features in the range of $10^3$-$10^4$. In this paper, we investigate a multi-scale AM process that can fabricate biomimetic structures with multi-scale features from micro- to macro- scales. Hence the ratio of the size span to the smallest geometry features could be in the range of $10^4$.

In the investigated multi-scale fabrication method, a novel micro-scale immersive surface accumulation (ISA) process is applied to add micro-scale surface structures on the macroscale and mesoscale 3D structures that are fabricated by the mask image projection based stereolithography (MIP-SL) process. Special fixtures were developed in order to integrate the macro- and meso-scale MIP-SL processes with the micro-scale ISA process. Two unique applications by using the developed method to fabricate biomimetic multi-scale objects have been demonstrated. Driven by the discoveries based on the multifunctional biological structures and the recent material advances, our multi-scale 3D printing process has the potential of enabling the fabrication of smart mechanical, fluidic and optical structures that require large dimensional size span and small geometric features at the same time. We envision such fabrication capability would enable novel multifunctional engineering components for various applications in the future.

**MACRO- AND MESO-SCALE MIP-SL PROCESS**

Stereolithography is the first commercially developed AM process. In the SLA process, controlled light source is used to selectively solidify photocurable polymers into two-dimensional (2D) layers. The fabricated 2D layers are then accumulated into 3D structures. For a given digital model with defined geometric information, the desired geometric shape of each layer is calculated. Accordingly, a laser or a 2D patterned mask image can be used to selectively cure photopolymer resin into defined shape. After one layer of liquid resin has been cured, liquid resin is refreshed into a thin film on the fabricated layers; hence the fabrication of the next layer can be repeated. The layer-based fabrication process enables SLA to fabricate complex shape with detailed features; in addition, the fabrication speed especially using mask image projection can be much faster than other 3D printing technologies.

Different mask image projection methods have been investigated for the SLA process [13, 17]. One of the most effective methods is the mask image projection based stereolithography (MIP-SL) based on a digital micromirror device (DMD). DMD controls the light reflection from a light source onto resin surface. In a macro-scale or meso-scale MIP-SL setup, a computerized...
system is used to process the computer-aided design (CAD) model and to generate the mask images of 2D layers. The DMD controller will then use the computed 2D mask images to control light exposure during the fabrication process. The projection images illuminated by the DMD need to be focused through optical lenses and to scale to a certain size that defines the fabrication extent. Such fabrication extent could be macro-, meso- or micro-scales. A clear and sharp image is eventually projected at the resin surface for proper exposure time to fabricate each 2D layer. After one layer of liquid resin is solidified, a computerized Z stage will move up the distance of a defined layer thickness and liquid resin will be refilled to enable the fabrication of next layer. The MIP-SL process allows different sizes of projection images to be used in the fabrication process, which provides flexibility on the achievable fabrication resolution by such a SLA process.

**Figure 2:** The diagram of the MIP-SL process.

The refreshing of liquid resin on fabricated layers is a critical step in the MIP-SL process especially for solid objects with large 2D layer sizes. When configuring the Z stage moving mechanism in the MIP-SL system, a design with considerable advantages is to use the bottom-up projection configuration [18]. Since the building platform and all the built layers need to be immersed inside the resin tank in the top-down projection configuration, the material consumption is much higher than the one in the bottom-up projection configuration. Furthermore, the building platform is raised by a layer thickness in the bottom-up projection configuration after each layer has been solidified. Hence a thin layer of liquid resin can be easily formed using the gap between the bottom of the resin reservoir and the previous layers. In comparison, an open refilling zone at the top exists in the top-down projection configuration, which usually requires a re-coating blade moving in the X direction. Hence, the bottom-up projection configuration can result in advantages including a simpler hardware setup to achieve precise thickness control, the use of significantly less resin, and much shorter re-coating time [19].

The projection areas of our macro- and meso-scale MIP-SL systems are designed to be 60 mm × 45 mm and 15 mm × 11.25 mm, respectively. The DMD chips used in our MIP-SL systems has 1024 × 768 micro-mirrors. Hence the pixel sizes of the macro- and meso-scale MIP-SL systems are 58.6 μm and 14.6 μm, respectively. The smallest geometry features that can be fabricated are around 50-100μm. However, since our MIP-SL setups cannot achieve the single pixel resolution, the micro-scale structures in the range of 5-50μm cannot be fabricated using the macro- and meso-scale MIP-SL systems. This is because, in the macro and meso-scale MIP-SL processes (refer to Fig. 3b-d), ultraviolet or visible light emission is used to trigger the polymerization process hence liquid resin can be solidified into solid. Three-dimensional structures are fabricated by precisely overlapping the input light energy of each projection pixels. The exposure curves of the liquid resin used in MIP-SL are quite nonlinear. It is rather difficult to achieve the presence of intensity threshold that allows the formation of light beam with dimensions that are considerably near the dimension of only a single or several pixels in the MIP-SL processes [20]. Besides the curing resolution in the XY plane, the cure depth of material in the Z direction is also a limitation for the micro-scale feature fabrication using the macro- and meso-scale MIP-SL process. Jacobs put forward a mathematical model for cure depth $C_d$ and exposure energy $E$, as shown in Eq. (1) [14]. By adjusting the percentage of light absorber, cure depth of material can be reduced from 250μm to 50 μm or even smaller for the micro-scale feature fabrication.

$$C_d = D_p \ln \left( \frac{E_{\text{max}}}{E} \right)$$

However, a micro-scale AM process is still needed in order to fabricate features in the range of 5-50 μm.

**Figure 3:** The experimental setup and test result of MIP-SL process. (a) The prototype machine of MIP-SL; (b) the CAD model of a mesoscale part with 100 μm pillar array; (c) one projection mask image (d) focusing image of the meso-scale SLA; and (e) the fabrication result of mesoscale part with micro-scale pillars using MIP-SL process.
MICRO-SCALE IMMERSIVE SURFACE ACCUMULATION PROCESS

The computer numerical control (CNC) accumulation is a non-layer-based additive manufacturing process [20]. Similar to the point-based and line-based CNC accumulation processes [21, 22], the surface-based CNC accumulation named immersive surface accumulation (ISA) has been developed to fabricated micro-scale features [23]. In the ISA process a light guide tool is used to provide light-resolution light source inside a resin tank to fabricate micro-scale features on a given surface. The ISA process contains an optical imaging system, a light guide tool movement system, and a light guide tool changing system. The optical imaging system of the ISA process is composed of a light source, a DMD chip, and a set of lenses. The illumination beam generated by the light source is collimated by a series of lenses. An illustrate diagram of the accumulation imaging system based on a DMD and a light guide tool is shown in Fig. 4. The light source is considered as a point source. Hence the light from it should firstly be collimated before the light reaches the DMD chip. By controlling the angle of the micro-mirrors in the DMD chip, different brightness of each pixel in the light beam can be obtained. An achromatic doublets lens with focus distant of 150mm is used to converge the light beams. In addition, a filter is used to block the light that has wavelengths other than 405nm. Finally, the collimated light goes through the 4× objective lens with focus distance of 15mm. The objective lens scales the projection image to the dimension that is desired. The distance between the objective lens and the collimated lens can be changed using a physical design as shown in Fig. 4. The dimension of the accumulation light beam used in our ISA prototyping system is 3.67mm ×2.75mm. Since the resolution of the used DMD chip is 1920 ×1080, the resolution of the light beam in our ISA system can reach 2.5μm per pixel.

![Diagram of the ISA process](image.jpg)

Figure 4: The diagram of the ISA process. (a) The layout of the ISA technology; and (b) the process planning to fabricate 3D micro-scale surface textures using the ISA technology.

The light guide tools in the ISA process have been constructed using both flexible optical fibers and optical acrylic rods. The light guide tools with different kaleidoscopic shapes and sizes can be used. They are immersed in liquid resin when fabricating different features on the surface of an inserted object. Finally, the light guide tool changing system is used to exchange different accumulation tools based on the 3D surface textures that need to be fabricated.

The immersive surface accumulation process is unique on the fabrication of 3D micro-scale structures. It is capable of fabricating complex geometries with feature sizes that are around 10μm. More importantly, since the ISA process does not use the layer-based fabrication approach, it can be used to add micro-scale surface structures on an object that has been pre-fabricated by some other manufacturing processes. Such fabrication capability by building-around-insert cannot be achieved by other micro-scale fabrication processes. Hence ISA could be an attractive solution for the fabrication of multi-scale structures by integrating with other AM processes due to the following two key features. First, the ISA process is an intrinsically three-dimensional additive manufacturing technique that can fabricate complex 3D features. Second, the ISA process can build-around-inserts. That is, it can fabricate micro-scale structures on the surface of a pre-existing object. By using different accumulation tools, both inside and outside surfaces can be added with desired surface structures.

Although the ISA process is versatile and flexible on fabricating microstructures, it has limitations on building macro- and meso-scale structures. Firstly, since the largest dimension of the focused 2D patterned beam is around 2mm, it is time-consumming to use the ISA process to fabricate large scale structures since the beam needs to scan the entire portion of each layer for a given model. Secondly, during the fabrication process, the surface curvature of the object is a critical factor in the ISA process, because the projection image should be focused at the place where the surface structures are to be added [24]. Macro- and meso-scale feature fabrication increases the difficulty for the ISA tool path planning and the collision may happen due to the actual dimension of the light guide tool and the pre-existing object shape. Consequently, to take advantage of the ISA’s micro-scale fabrication capability and the MIP-SL’s fast fabrication speed, it is beneficial to integrate the MIP-SL and ISA processes into a multi-scale AM process that can fabricate 3D objects with feature sizes varying from macro-, meso-, and micro-scales.

INTEGRATION OF THE MIP-SL AND ISA PROCESSES

The MIP-SL process is highly efficient in building objects with features that are in macro- and meso-scales; and the ISA process is capable of fabricating micro-scale features on the surface of a pre-existing object. Hence an ideal multi-scale fabrication method is to integrate both MIP-SL and ISA processes to fabricate complex macro- and meso-scale geometry features together with high resolution micro-scale surface features. In order to integrate the MIP-SLA and ISA processes, we firstly build an object using the macro- or meso-scale MIP-SL processes. We then plan the micro-scale textures on the object surface based on the digital model of the fabricated macro-scale object. Depending on the desired functions to be added on the surface, the CAD model of micro-scale features can be designed. We further select appropriate light guide tool based on the shape of the micro-scale surface.
textures. Based on the shape and size of the selected light guide tool, the tool path of the light guide tool is planned. Accordingly, the movement of the light guide tool is used in the ISA process to fabricate micro-scale textures on the given object surface (refer to an illustration case shown in Fig.5).

![Image](image)

**Figure 5:** The diagram of the integration of the MIP-SL and ISA processes. (a) The multi-scale structures; (b) the fabrication of macro- and meso-scale structures by the MIP-SL process; (c) the fabrication of micro-scale surface textures on object surface by the ISA process; and (d) the fabrication of micro-scale textures on macro-scale object surface by the ISA process in multiple directions.

To fabricate micro-scale features on an insert object surface, the ISA process needs to get the relative position of the pre-existing object relative to the light guide tool. The initial position of the light guide tool is important since the deformation of the PDMS film on the tip of the light guide tool may have a deformation larger than 100 μm when it is under external pressure. Such deformation may be significantly larger than the thickness of 3D micro-textures that need to be fabricated. Consequently, the micro-scale feature fabrication will not be successful since no gap exists between the light guide tool and the inserted object surface. To eliminate the external pressure during the tool movement, it is critical to identify the initial position of the pre-existing object when it is mounted on the building platform. As the light guide tool moves down, the distance L between the tip surface of the tool and the insert object surface decreases. Hence the volume of liquid resin between these two surfaces will be reduced. Accordingly, the color of liquid resin capture by the added digital camera becomes lighter with the decrease of distance L. Taking advantage of image processing, the distance between the object surface fabricated by the MIP-SL process and the light guide tool can be determined based on computing the color difference of liquid resin during the movement of the light guide tool. The gap distance that can be controlled using our image-processing-based position detection method is 20μm.

**TEST CASES AND DISCUSSION**

Two test cases were performed to verify the capability of the developed multi-scale AM process including scaffolds with micro-scale textures inside culture plates for cell study and the superhydrophobic micro-textures inspired by salvinia molesta leaves. These test cases demonstrate that textures with different types of geometries can be successfully fabricated by the presented multi-scale fabrication method. In addition, different materials are used in the two test cases, which demonstrates the immersed surface accumulation process is general on the used materials and can be used in building multi-material features.

**Test case 1: Scaffold with 3D micro-textures inside a culture plate**

Three-dimensional micro-scale scaffolds are critical for long term cell and embryoid body culture. For the traditional injection molding process, only simple 2D mesh can be fabricated. Such limited shape complexity restricts the understanding of the cell growth in various 3D environments in medical research. To address the problem, we applied our multi-scale AM process to fabricate the designed micro-scale features inside a standard 24-wells cell culture plate. Such a plate can be 3D printed by the MIP-SL process or pre-fabricated by the injection molding process. As shown in Fig. 6 and 7, several cell culture scaffolds with complex geometric shapes were built to verify the capability of our multi-scale AM process.

![Image](image)

**Figure 6:** The design and fabrication of the scaffold with 3D micro-scale concave textures that were printed using the integrated MIP-SL and ISA process. (a) Design of multi-scale artificial cell culture environment; (b, e) the design and fabrication of micro-scale scaffold; (c, d) the design and fabrication of meso-scale scaffold wall; (f) the integration of ISA printing for micro-scale scaffold printing; (g, i) the microscope image of without and with 3D printed scaffold; and (h, j) the fabrication results without and with micro-scale scaffold.
The original cell culture well has a diameter of 15.6 mm, which consumes a significant amount of cells and chemical solutions that may be expensive. To reduce the culture size, we design our artificial cell culture environments as shown in Fig. 6a. The artificial cell culture environment contains the outer wall of scaffold, which prevents the leaking of culture media including both cells and chemical solutions (refer to Fig. 6c). The height of such wall is 15 mm and the total XY size is 10 × 10 mm. We first used the meso-scale MIP-SL process to build such wall using the biocompatible 100% PEGDA solution (refer to Fig. 6d). Inside the wall we designed 3D scaffold with micro-scale textures (refer to the top view of a cell in the culture plate). The CAD model of the micro-scale scaffold is shown in Fig. 5b. The total height of the scaffold wall is 150 mm and the total XY size of the scaffold is 2.5 mm × 2 mm. In the test, the light guide tool based on the optical acrylic rod is used to build the new scaffold wall inside the artificial culture environment printed by the MIP-SL process (refer to Fig. 6e). To achieve high resolution surface quality required by the micro-scale scaffold, the slicing thickness of the concave textured scaffold is set at 3 μm per layer. The setup during the fabrication process and special fixture are shown in Fig. 6f. The fabrication results of the artificial cell culture environment with and without 3D micro-scale scaffold are shown in Fig. 6h and 6i, respectively. And the microscope images of the artificial cell culture environment with and without 3D micro-scale scaffold are shown in Fig. 6g and 6j, respectively. The added 3D scaffolds instead of a flat surface can provide significant advantages for the long-term cell growth study.

In order to better understand the cell culture growth on the micro-textured scaffold, we also designed and fabricated scaffolds with multiple geometric shapes. As shown in Fig. 7a, the concave scaffold of anti-tetrahedron shape was built with one bar 30 μm in the center to inhibit the forming of spheroids. To study the effect of dimension on the micro-scale concave scaffold, the printed scaffold with textures in multiple dimensions were designed as shown in Fig. 7b. Furthermore, as shown in Fig. 7c, micro-scale scaffold with different shapes can also be designed and fabricated. The microscopy images of the 3D printed scaffolds with 3D concave textures are shown in Fig. 7d. In Fig. 5b, the artificial cell culture scaffold with the macro-scale design was fabricated using our multi-scale AM process. The top view of such scaffold is shown in Fig. 6f. In Fig. 5b, the artificial cell culture scaffold with the macro-scale design was fabricated using our multi-scale AM process.

**Material preparation in test case 1:** The PEGDA based hydrogel solution was used in the test that is biocompatible. This hydrogel solution is made by 60 wt% percentage poly(ethylene diacrylate) (PEGDA, Mw 700, Sigma-Aldrich). The visible light photoinitiator (Iracure 819, BASF) was used at a concentration of 1 wt% to induce polymerization by the free radicals. The photoinitiator was firstly fully dissolved in the phosphate buffered saline (PBS) before adding to the hydrogel solution.

**Test case 2:** A lotus shaped 3D structure with superhydrophobic micro-textures

Salvinia molesta is a plant in South America that lives on water. Its leaf has multi-scale eggbeater shaped structures that can keep water droplet perfectly round on the top surface of the leaf (superhydrophobic effect). We can bio-mimic such multi-scale structures and fabricate the superhydrophobic structures on the surface of a macro-scale lotus shaped object that is 3D printed by the MIP-SL process. Hence we can modify the interface performance of the macro-scale object with the micro-scale eggbeater structures. Both the macro-scale lotus shape and the micro-scale eggbeater structure were designed using Solidworks as shown in Fig. 8b and Fig. 8c, respectively. The designed multi-scale object, as shown in Fig. 8a, has a dimension of 30 mm × 30 mm, and the height of the eggbeater shaped structures is 800 μm while the four hairs of the eggbeater structure is 30 μm in their diameters.

Using our multi-scale AM process, we firstly printed the lotus shaped object using the macro-scale MIP-SL process and S1500 resin (from Envisiontec Inc.) as shown in Fig. 8d. The layer thickness of the 3D structure is 100 μm and the exposure time is 5 s per layer. We then printed biomimetic eggbeater structures on the surface of the lotus shaped object using the ISA process (refer to Fig. 8e). We developed composite material by mixing liquid resin with multi-wall carbon nanotubes (MW-
CNT) in fabricating the superhydrophobic structures. The cure depth of the composite material is 30μm per layer using 20s light exposure. Fig.8f shows the setup in the ISA printing process to fabricate bioinspired eggbeater structures. Fig.8h shows the printing result of the lotus-shaped object using the macro-scale MIP-SL process; and Fig.8k shows the printing result after adding biomimetic eggbeater structures on the lotus shaped object. Fig.8g and 8j show the scanning electron microscope (SEM) images of the object surface without and with bio-mimetic structures, respectively. After adding one water droplet on the modified surface, we can see the superhydrophobic effect on the surface with biomimetic micro-scale surface structures (refer to Fig.8i and Fig.8l). The developed multi-scale 3D printing approach can be used to quickly add micro-scale structures on a large surface to modify its surface functions [25].

**Material preparation in test case 2: MW-CNT based photocurable composite** was used in the test. The E-glass resin purchased from Envisiontec Inc. was directly used. We mixed 3% MW-CNT (Bucky USA, Inc) with E-glass resin for 2 hours under a magnetic stirring and another 30 minutes under the ultrasonic bath. The mixed composite was then degassed in the vacuum before the ISA process.

**CONCLUSIONS**

Creatures in nature show interesting functions with multi-scale hierarchical structures. Smart structure design inspired by the hierarchical structures in nature can benefit future engineering systems. To fabricate multi-scale biomimetic structures, a promising manufacturing approach is to integrate multiple additive manufacturing processes to fully utilize their respective advantages. In the paper, we presented our investigation on a multi-scale 3D printing method by integrating the macro- and meso-scale projection based stereolithography process and the micro-scale immersed surface accumulation process. We discussed the basic idea, hardware setup, and process development of the multi-scale 3D printing process. The process planning of both projection based stereolithography and immersed surface accumulation and the curing characteristics of used photocurable materials were studied. Two types of multi-scale bioinspired structures were tested to demonstrate the effectiveness of the developed fabrication method. By adding micro-scale features on an object surface, the functional performance of the macro-scale surface can be effectively modified. Our newly developed multi-scale and multi-material AM process presents a fabrication tool that could open up broad prospects for biomimetic study and enable a wide range of applications that may benefit from biomimetic structures.

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