ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

A Facile Multi-Material Direct Laser Writing Strategy

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Supplementary Text

3D Model Preparation

The computer-aided design (CAD) software Solidworks (Dassault Systémes) was first utilized to generate 3D models for each material layer of all printed components. The CAM software DeScribe (Nanoscribe GmbH) was then used to convert CAD models into writing path scripts, which define the laser path to produce 3D structures from slicing, hatching, and contour lines. For DiLL mode prints of negative channel mold files, scripts were defined with 1000-nm-thick slices and 500 nm between hatching and contour lines, whereas writing path scripts for multi-material components were defined with 300-nm-thick slices and 300 nm between hatching and contour lines. The ellipsoidal voxel size was also defined in these scripts, with a 500 nm diameter and 3.5 aspect ratio voxel for channel master molds and a 300 nm diameter and 3.5 aspect ratio voxel for multi-material components. Finally, scripts for each material sub-component were assembled into a single print file and CAM simulations were generated to ensure the registration of distinct material layers. The files were then uploaded for DLW printing to the CAM software NanoWrite (Nanoscribe GmbH), which is interfaced with the Nanoscribe DLW printer.

Conventional Multi-Material DLW Technique

As an experimental control for comparison of the μFMM-DLW strategy, a conventional multi-material DLW strategy was used to generate multi-tier alignment structure components. To fabricate such components, a circular borosilicate glass substrate was first prepared for printing by cleaning with washes of acetone and IPA, and drying with inert N_2 gas (**Fig. S1a**). The substrate was then mounted, and approximately 100 μL the preliminary liquid photopolymer, IP-L 780 (Nanoscribe, GmbH), was drop cast on the top side of the glass substrate (**Fig. S1b**), while immersion oil was drop cast on the back side of the substrate. Substrates were loaded into the printer in the oil-immersion mode. Fabrication of the primary material layer was conducted with DLW using a 63$\times$ objective lens (**Fig. S1c**). To eliminate excess uncured photomaterial, substrates were then removed from the DLW printer (**Fig. S1d**) and placed in a developing bath of 15 mL of PGMEA for 12 minutes (**Fig. S1e**). Devices were then transferred to a bath of 15 mL of IPA for 2 minutes to wash the substrate and clear away the developing solvent, and then dried with a gentle stream of N_2 gas (**Fig. S1f**). To prevent detachment of the printed components from the glass surface, the aforementioned PGMEA and IPA development steps were performed under quasi-static fluidic conditions (*e.g.*, without agitation or sonication, *etc.*). The substrate was then prepared in a similar manner as before, with approximately 100 μL of the secondary photopolymer, Rhodamine-B-dyed IP-L 780, drop cast over the primary structure (**Fig. S1g**), and immersion oil drop-cast on the underside of the substrate. After loading the substrate into the printer, the mounting stage was manually translated to align the central point of the writing area to the central point of the previously fabricated structure. Similarly, the coordinate system of the printer was rotated to accurately match the angular offset of the previously fabricated component (**Fig. S1h**). Once fully aligned, the secondary material structures were fabricated with DLW (**Fig. S1i**). Subsequently, the substrate was removed (**Fig. S1j**), developed with PGMEA (**Fig. S1k**), washed with IPA, and dried with N_2 gas (**Fig. S1l**).

Channel Mold Fabrication

The μFMM-DLW strategy relies on the preliminary fabrication of a customized microfluidic channel mold for subsequent PDMS replication. Negative master molds were generated using the DiLL configuration of DLW. The straight channel molds were designed to be 5 mm long with a semi-ovular cross section that was 100 μm tall and 100 μm wide (**Fig. S2a**), and were fabricated with the photoresist IP-Dip (Nanoscribe GmbH) and a 25$\times$ objective lens on a 25mm $\times$ 25mm Si substrate. The light path was set using 1-μm-thick slices (**Fig. S2b**) and 0.5-μm-thick hatches (**Fig. S2c**) to generate the overall structure. Furthermore, to reduce overall fabrication time, the mold was designed with 10 contour layers and a triangular scaffolding hatching (**Fig. S2d**). Following fabrication, the substrate was removed from the DLW printer and developed in serial washes of PGMEA (15 minutes) and IPA (2 minutes).

Fabrication Time Comparison

In addition to material registration accuracy, another metric of comparison between conventional multi-material DLW and μFMM-DLW is the overall fabrication time. Fabrication experiments with two-material components resulted in an average total time of 61$\pm$7 min for the conventional techniques and 22$\pm$1 min for the μFMM-DLW approach (**Fig. S3**). Although the times for the DLW printing and substrate handling steps remained relatively consistent between the two strategies, several other fabrication steps led to significant disparities. In particular, the elimination of the protocols associated with manual alignment (*e.g.*, locating the print area, manually setting rotational (θ) and Cartesian coordinates – *see also* **ESI Movie S2**) resulted in a considerable reduction in process labor and time (*i.e.*, by $\sim$21$\pm$7 min) for the μFMM-DLW strategy. In addition, because of the scaling-induced benefits of microchannel-based fluid flow, the development steps for μFMM-DLW also required approximately 70% less time than their conventional counterparts. One caveat, however, is that the steps associated with initial substrate preparation and loading and final substrate removal for μFMM-DLW required slightly more time ($\sim$33%) than the conventional methods. In combination, these results suggest that utilizing μFMM-DLW to reduce the overall fabrication time would be increasingly beneficial as the number of distinct materials is increased. Notably, μFMM-DLW manufacturing of the five-material DNA-inspired structures (*e.g.*, **Fig. 3a**) corresponded to total fabrication times of approximately 55 min – less time than required for conventional multi-material DLW of the two-material systems (**Fig. S3**).

Supplementary Figures

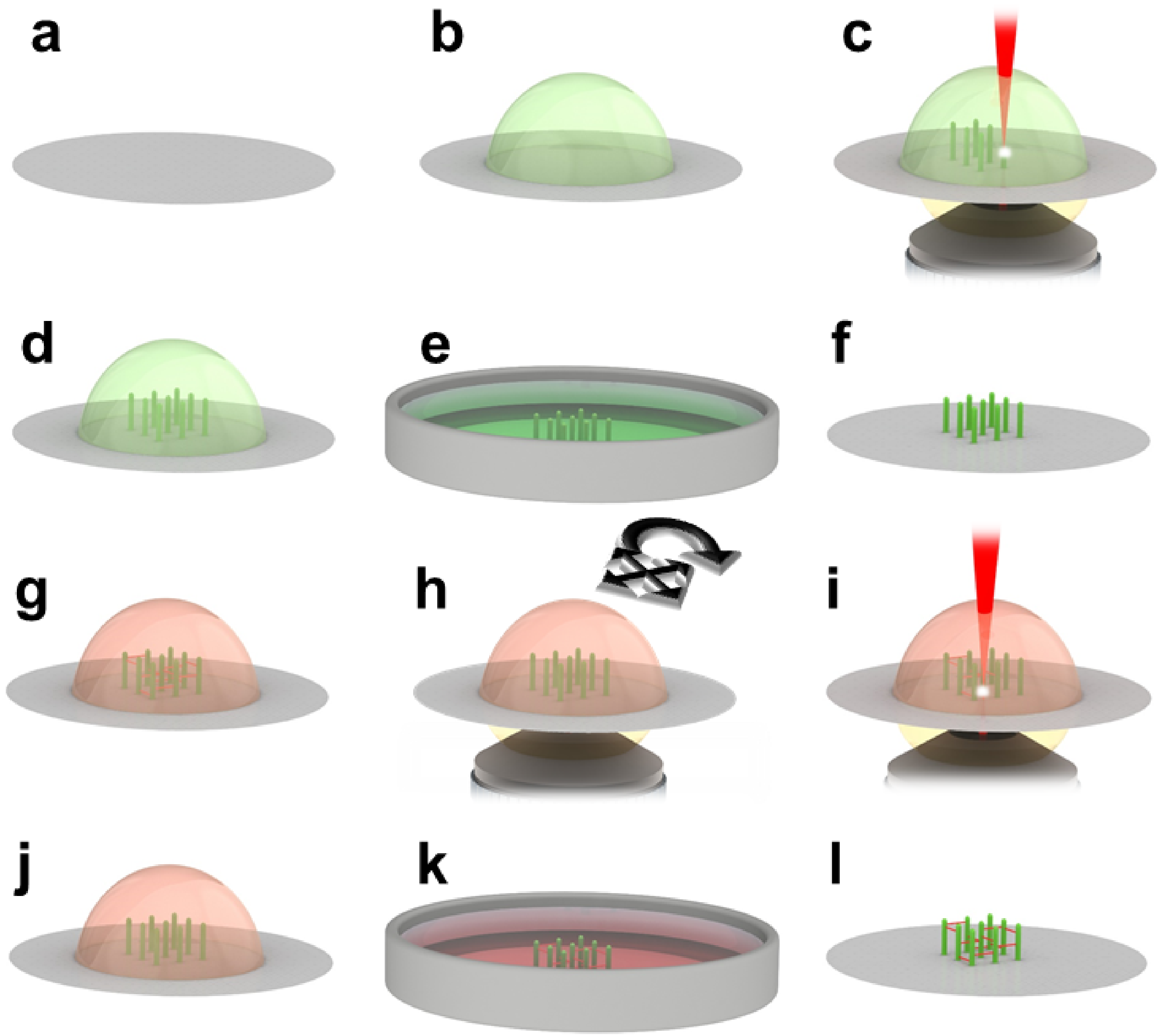

Fig. S1 Conceptual illustrations of a “conventional" multi-material DLW protocol. (**a**) Glass substrate. (**b**) Drop-casting of first photomaterial (*green*) onto the glass surface. (**c**) DLW fabrication of the first material structures using the oil-immersion mode configuration. (**d**) Removal of the completed prints of the first material structures from the DLW printer. (**e**) Development of the first photomaterial. (**f**) Completed first material component. (**g**) Drop-casting of a second photomaterial (*red*) onto the substrate. (**h**) Loading of the substrate into the DLW printer, followed by manual rotational and lateral alignment of the writing area. (**i**) DLW fabrication of the second material structures using the oil-immersion mode configuration. (**j**) Removal of the substrate from the DLW printer. (**k**) Development of the second photomaterial. (**l**) Completed two-material system.

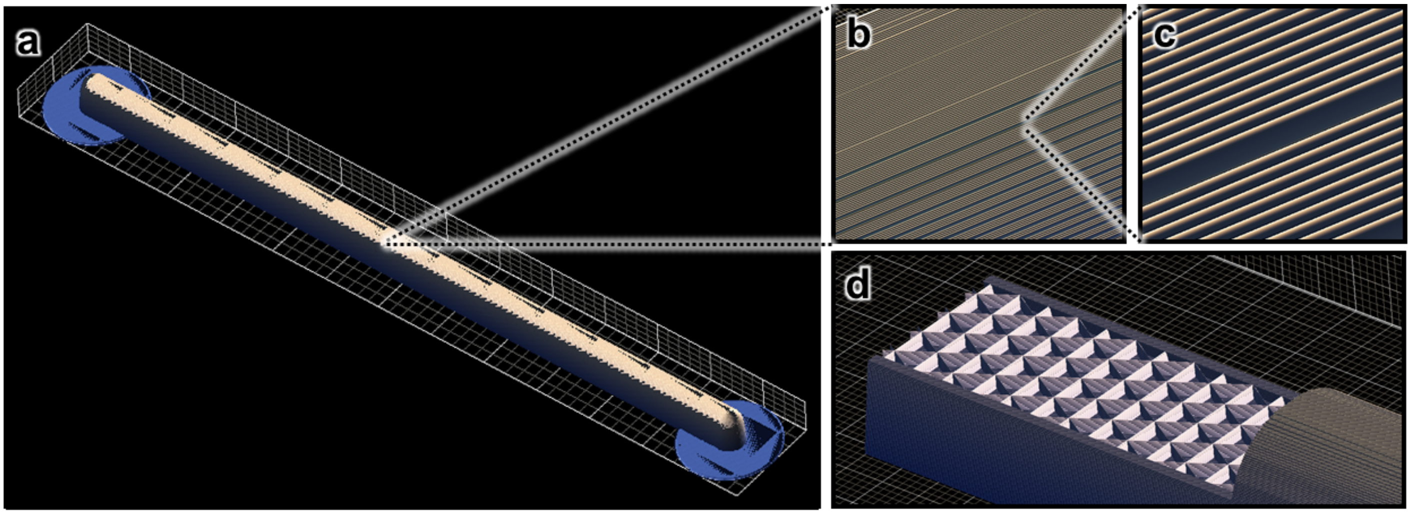

Fig. S2 Images from CAM simulations of microchannel negative master mold DLW fabrication. (**a**) The negative microchannel master mold with circular areas (*blue*) to direct inlet and outlet placement. (**b**) Expanded view of the 1-μm-thick layer slices that comprise the laser writing path. (**c**) Expanded view of the 0.5-μm-thick hatching for the laser writing path of each layer within the mold. (**d**) Expanded sectional view of the 10 contours and triangular scaffolding hatching for the laser writing path of each layer within the mold.

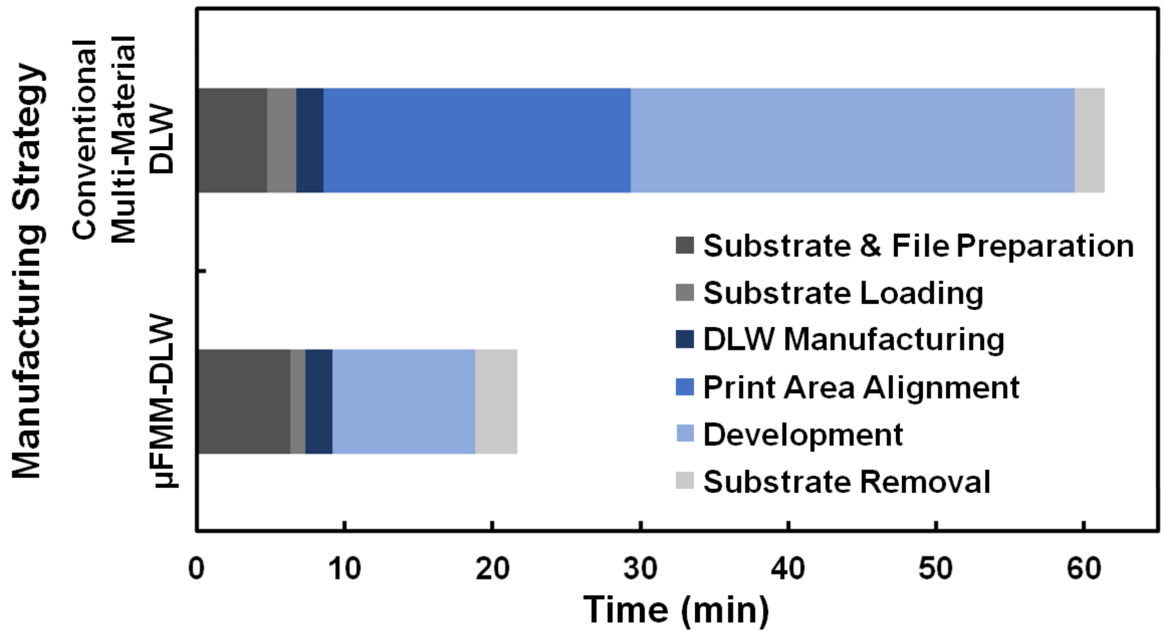

Fig. S3 Results for average time required to execute distinct fabrication steps corresponding to conventional multi-material DLW and μFMM-DLW.

Supplementary Movie Captions

Supplementary Movie S1. Results for computer-aided manufacturing (CAM) simulations (*top*) and corresponding μFMM-DLW fabrication (*bottom*) for a five-material DNA-inspired microstructure. Video Speed = 5×

Supplementary Movie S2. The manual alignment protocol utilized in the conventional multi-material DLW technique. The procedure includes four steps prior to DLW fabrication of a second photomaterial: (*i*) manually searching for the previously fabricated component; (*ii*) manually aligning the rotational orientation (θ) of the printing coordinates to the previously fabricated component; (*iii*) manually aligning the X- and Y-directional orientation to the previously fabricated component; and (*iv*) aligning the Z position of the orientation to the glass interface. Video Speed = 5×