TOWARDS DETERMINISTIC LATERAL DISPLACEMENT-BASED CONTINUOUS-FLOW MICROFLUIDIC PARTICLE REACTORS *VIA* DIRECT LASER WRITING

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ABSTRACT

Multi-stage fluidic reaction schemes for suspended particles (*e.g.*, micro/nanospheres, cells, bacterial species, and extracellular vesicles) underly a diversity of chemical and biological applications. Conventional methods for executing such protocols can be exceedingly time, labor, and/or cost intensive. Microfluidic strategies can address these drawbacks; however, such technologies typically rely on clean room-based microfabrication that suffer from similar deficits for manufacturing the chips. To simultaneously overcome these challenges, here we explore the use of the submicron-scale additive manufacturing approach, "Two-Photon Direct Laser Writing (DLW)", as a means for fabricating micro-fluidic "Deterministic Lateral Displacement (DLD)" arrays capable of passively guiding suspended particles across discrete, adjacent flow streams—the fundamental capability of continuous-flow multi-stage particle microreactors. Experimental results from microfluidic experimentation with 5 μm-in-diameter fluorescent particles revealed effective particle transport across flow streams, with 87.5% of fluorescent peaks detected in the designated, opposing outlet following the DLD array. These results suggest utility of the presented approach for micro- and nanoparticle-based microfluidic reactors targeting wide-ranging chemical and biological applications.

KEYWORDS

Additive Manufacturing, Direct Laser Writing, Deterministic Lateral Displacement, Microfluidics, 3D Printing

INTRODUCTION

Continuous-flow particle-based microreactors are uniquely suited for biological and chemical applications that are predicated on multi-stage fluidic reaction schemes (*e.g.*, layer-by-layer depositions, sandwich assays), including for micro/nanoparticle coatings and preparation of advanced functional materials [1-6]. To take advantage of these scaling-induced benefits, such as low sample and reagent volumes as well as high control of micro-environmental conditions, numerous researchers have explored strategies for passively transporting micro/nanoparticles across parallel flow streams [7]. Previously, our group reported a continuous-flow micropost array railing approach [8] and demonstrated its use for a six-stage cytokine-detection sandwich assay on microparticle substrates [9]. Unfortunately, this approach requires the arrayed posts to match the size of the target particles, thereby restricting the size of the particles that can be processed to the minimum feature size of the manufacturing technology [8,9]. In contrast, "Deterministic Lateral Displacement (DLD)" technologies—continuous-flow fluidic systems comprising micro/nanoposts arrayed at an angle with respect to the primary flow direction—circumvent the aforementioned issue by enabling the handling of suspended particles that are much smaller than the size of the arrayed posts [10-15]. Unfortunately, conventional methods for manufacturing such microfluidic systems are generally time, cost, and labor intensive [16,17]. Thus, alternative approaches that address these issues are in critical demand.

CONCEPT

Previously, we demonstrated the use of the submicron-scale additive manufacturing (or "three-dimensional (3D) printing) technology, "Two-Photon Direct Laser Writing (DLW)", for printing vertically stacked DLD arrays inside of microfluidic channels to enable size-based particle sorting within a single flow stream [18]. In this work, however, we investigate the potential for DLW-based DLD arrays to instead serve as a means for passively guiding suspended microparticles into adjacent, discrete flow streams—a key proof of concept for continuous-flow particle-based microreactors. To elucidate this capability, we use DLW to print a negative master mold (**Fig. 1a**), which we then replicated to produce an enclosed polydimethylsiloxane (PDMS)-on-glass microfluidic device. The overall concept is presented in **Figure 1b**, which entails designing the geometric dimensions of the system—*i.e.*, the gap spacing between microposts (G) and the angle of the array (θ) with respect to the flow direction—to ensure that the diameter of the target particles is larger than the critical diameter (D_C) based on the DLD array. Researchers previously reported [12] an empirical formula for these relationships as:

$$D_C = 1.4G \times tan(\theta)^{0.48} \quad (1)$$

Importantly, the height of the posts is not present in *Equation 1*, as the height only affects the throughput, and not the railing capability.

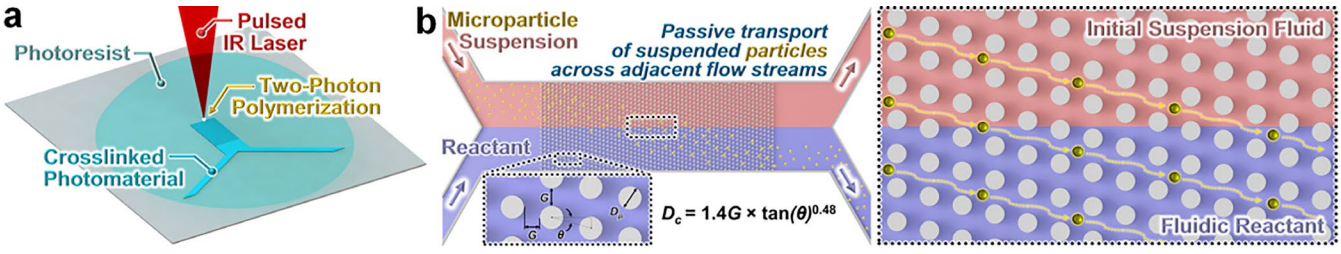

Figure 1: *Conceptual illustrations of a "Direct Laser Writing (DLW)"-based "Deterministic Lateral Displacement (DLD)" array continuous-flow microfluidic particle reactor.* ***(a)*** *DLW of the microfluidic system master mold onto a Si wafer.* ***(b)*** *Microfluidic system overview. Suspended microparticles are inputted via the top left channel, enter the DLD array to be passively guided into the distinct, adjacent "reactant" flow stream, and outputted via the bottom right channel. The critical (i.e., minimum) diameter (D_C) of suspended particles that can be transported is based on the gap spacing (G) and the angle of the array (θ) with respect to the primary flow direction.*

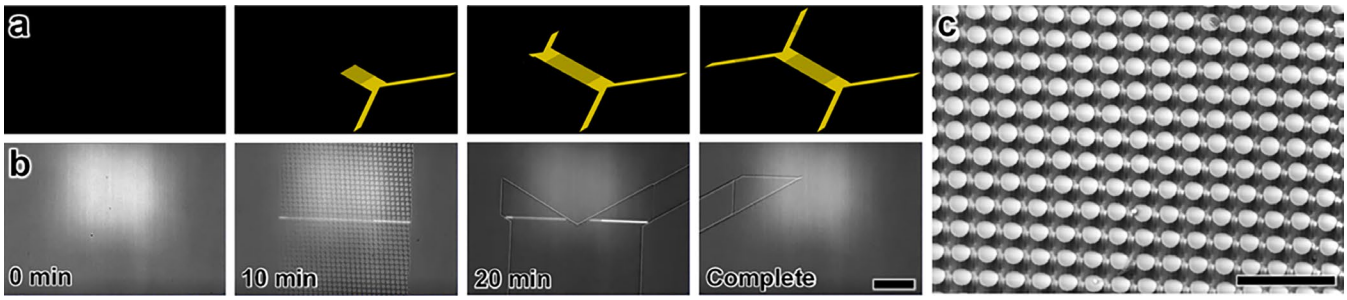

Figure 2: *Fabrication Results.* ***(a,b)*** *Sequential images of* ***(a)*** *computer-aided manufacturing (CAM) simulations, and* ***(b)*** *the corresponding DLW-based printing process of the mold onto a Si wafer. Scale bar = 250 μm.* ***(c)*** *SEM micrograph of print results. Scale bar = 200 μm.*

To avoid stiction-based print failures, the height of the posts, and therefore the channel, was limited to 20 μm. As a result of the unique DLD geometry and its relation to the flow direction, suspended microparticles will be guided by the DLD array away from their initial suspension fluid into a distinct, adjacent reactant fluid (**Fig. 1b – *expanded view***).

MATERIAL AND METHODS

Direct Laser Writing (DLW) of the Deterministic Lateral Displacement (DLD) Array Negative Mold

The overall DLW-based strategy for manufacturing the fully enclosed PDMS-on-glass microfluidic devices is based on a methodology reported previously by our group [19]. Briefly, the negative master mold of the microchannels and DLD array were modeled using the computer-aided design (CAD) software, SolidWorks (Dassault Systems, France), and then exported as an STL file. This file was then imported into the computer-aided manufacturing (CAM) software, DeScribe (Nanoscribe, Karlsruhe, Germany), for slicing and generation of point-by-point, layer-by-layer laser writing path. The cross section of the microchannel was designed with a trapezoidal shape for easier removal of PDMS off the mold [18,19].

A Si substrate (25 mm × 25 mm) was rinsed with acetone and isopropyl alcohol (IPA) and then dried with N_2 gas before being loaded into a Plasma Cleaner (Pie Scientific, Union City, CA) for 30 min at 75 Watts. A drop of IP-Q photoresist (Nanoscribe) was manually dispensed onto the Si substrate, and then both the substrate and resist were heated on a 65 °C hot plate for 10 min. This substrate was then loaded into the Nanoscribe Photonic Professional GT printer in the Dip-in Laser Lithography (DiLL) configuration with the 10× objective lens. Following the DLW printing process, the fabricated mold was developed *via* successive immersion in propylene monomethyl ether acetate (PGMEA) and IPA, and lastly, dried with N_2 gas.

PDMS-on-Glass Microfluidic Device Fabrication

A 10:1 mixture of PDMS (Slygard 184, Dow Corning, Corning, NY) was poured over the master molds and placed on a hot plate at 65 °C for approximately one hour. The nearly cured PDMS was then carefully peeled from the molds and punched with 0.75 mm-in-diameter ports at inlet and outlet locations. The PDMS was rinsed with IPA and DI water, and then O_2 bonded to 30 mm circular borosilicate glass substrates (#1.5, Bioptechs Inc. Butler, PA). The sealed device was then placed on a hot plate at 65 °C overnight to promote the integrity of the PDMS-to-glass bond adhesion and to ensure full curing of the PDMS.

Experimental Characterization

Scanning electron microscope (SEM) images were captured using a TM4000 Tabletop SEM (Hitachi, Tokyo, Japan). Microfluidic experiments were performed using the Fluigent Microfluidic Control System (MFCS). Two solutions/suspensions were prepared for testing: (*i*) a buffer solution of 2% Tween 20 in DI water for input into the bottom port, and (*ii*) a microparticle suspension comprised of 2% Tween 20 in DI water and 1% v/v 5 μm-in-diameter microparticles with fluorescence at 500 nm. Fluorescence results were obtained *via* an inverted fluorescence microscope (Axio Observer.Z1, Zeiss, Germany) connected to a camera (Axiocam 503 Mono, Zeiss). To quantify the locations of the suspended microparticles within the images, the fluorescence micrographs were processed using ImageJ (NIH, Bethesda, MD) to measure the intensities of fluorescence at the locations of the microparticles upon entry, within the DLD array, and upon exit [20].

RESULTS AND DISCUSSION

DLW-Based Fabrication

CAM simulations and corresponding micrographs of the DLW printing process for the molds are presented in **Figure 2a** and **2b**, respectively. Fabrication results of the DLD array in PDMS revealed effective replication of the G = 7.5 μm, θ = 0.1 rad geometries (**Fig. 2c**), which corresponds to D_C = 3.5 μm (*Eq. 1*). Due to shrinkage phenomena associated with both the print and PDMS, the initial model was designed to ensure such size changes would still result in a final device suitable for handling target 5 μm-in-diameter particles. To achieve the intended G = 7.5 μm, we originally designed the posts with a diameter of 22 μm and with G = 5.5 μm, which resulted in the final device exhibiting 20 μm-in-diameter posts, thereby enlarging G to the intended 7.5 μm (**Fig. 2c**).

Microparticle-Based Microfluidic Experimentation

To investigate the functionality of the DLD device for the passive transport of suspended particles across adjacent flow streams, we performed continuous-flow microfluidic experiments with suspended 5 μm-in-diameter particles while monitoring the positions of the particles with respect to the two flow streams using fluorescence imaging. The fluorescence imaging of the particles as they traveled through the DLD and across flow streams revealed several key trends. First, similar to that reported in prior works [16,18] we observed a degree of particle clogging at the beginning of the DLD array, as evidenced by the brighter regions at the entrance of the DLD arrays (**Fig. 3a**). The surfactant, Tween 20, which was mixed into the particle suspension solution, mitigated some of the clogging and allowed for the majority of inputted particles to enter the array and exit the array (**Fig. 3b,c**). In the middle regions of the array, we observed that, while the overall path of the suspended microparticles appeared to trend toward the adjacent flow stream, the particles did appear to backtrack in part at various points (**Fig. 3b**). Although such behavior suggests that the D_C was too close to the diameter of the inputted particles, it was not detrimental to the overall particle guidance efficacy as the majority

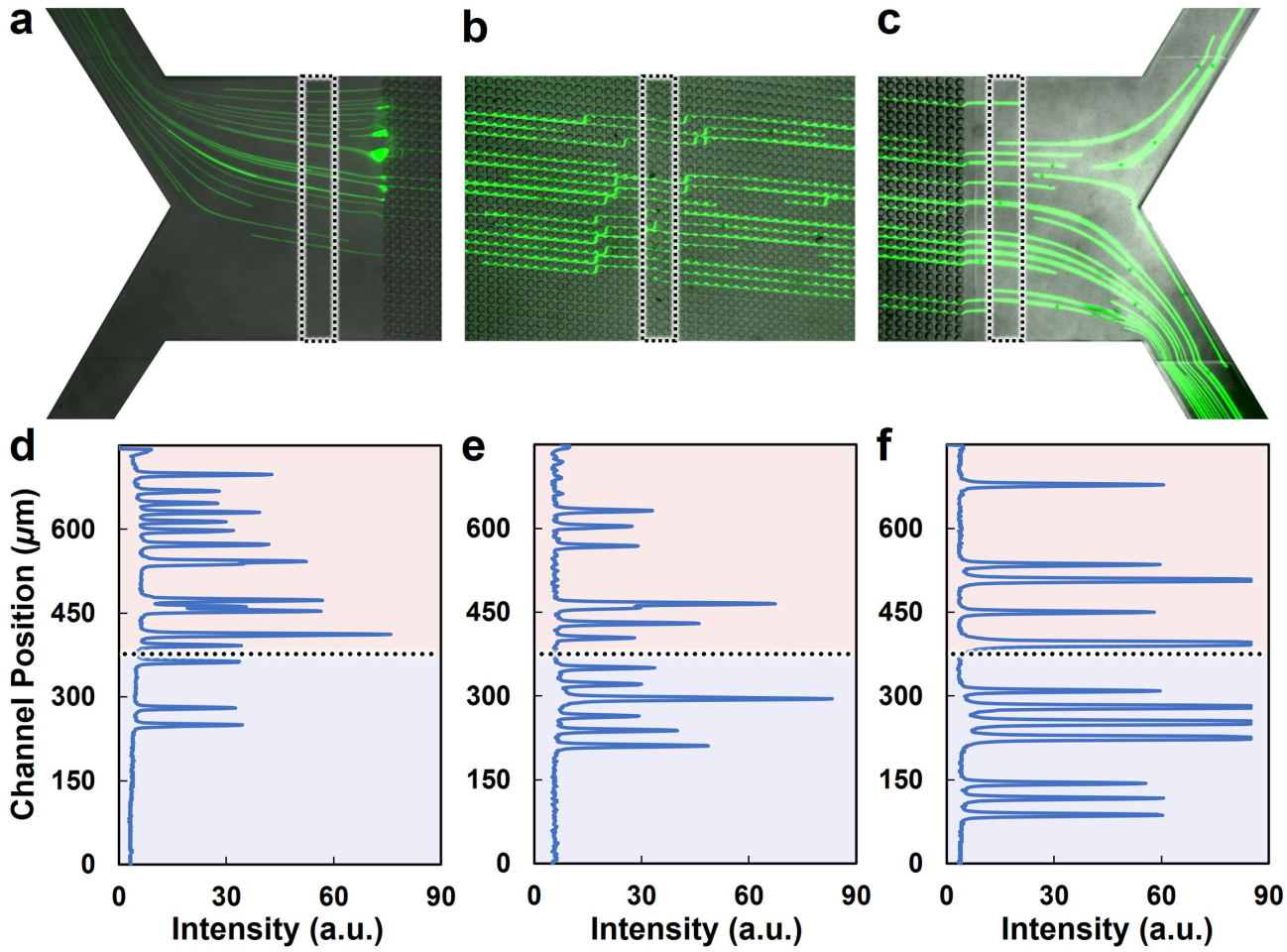

Figure 3: *Microfluidic experimental results for DLD-based microparticle transport of suspended 5 μm-in-diameter fluorescent particles across discrete, adjacent flow streams. Fluorescence micrographs* ***(a–c)*** *and corresponding quantified fluorescence intensity results along the width of the channel* ***(d–f)*** *for regions of interest (dotted boxes):* ***(a,d)*** *preceding the entrance of the DLD array,* ***(b,e)*** *in the middle of the DLD array, and* ***(c,f)*** *after exiting the DLD array.* ***(d–f)*** *Dotted line denotes the middle of the microchannel, separating the microparticle suspension fluid (light red) from the 'reactant' fluid (light purple).*

of inputted particles successfully traversed into the adjacent flow stream (**Fig. 3c**).

To evaluate the change in location of the particles within the device, we captured fluorescence images at entrance, middle, and exit regions of the DLD array as particles flowed through and quantified the fluorescence intensity along the channel at each region (**Fig. 3d–f**). These results revealed a marked shift from the majority of peaks in the top region when initially entering the DLD array (**Fig. 3d**) to the bottom region after exiting the array (**Fig. 3f**). To further interrogate the efficacy of the DLW-based DLD array for microparticle transport across flow streams, we also captured and quantified fluorescence images at both of the inlets and outlets. These results revealed a significant shift from before (**Fig. 4a,b**) and after (**Fig. 4c,d**) the DLD array. In particular, while we observed 100% of fluorescent peaks associated with the initial suspension fluid inlet (as expected) (**Fig. 4a**), we found less than 15% of the fluorescent peaks remained after the array at the top suspension fluid-associated outlet (**Fig. 4c**) as the majority of the peaks were instead at the surrogate 'reactant'-associated outlet (**Fig. 4d**). These results suggest utility for the DLW-based DLD array for passive guidance of suspended microparticles into an adjacent flow stream.

CONCLUSION

Here we investigated the potential of leveraging DLW to enable DLD arrays capable of passively transporting suspended microparticles across distinct, adjacent flow streams. The fabrication and experimental results suggest efficacy for this approach, which could offer a novel pathway to new classes of continuous-flow, multi-stage particle reactors for target applications [21,22]. In future works, this strategy could be extended to design systems with multiple DLD arrays in series and additional fluidic inputs to passively execute multi-stage fluidic reaction schemes. Because the DLW-based manufacturing strategy bypasses the need for clean room-based photolithography protocols, the presented approach can also reduce the time, cost, and labor requirements associated with past works [8,9].

Although DLD arrays are highly effective at particle sorting by size, in their current form, they present multiple issues in that they have limited throughput and can be prone to clogging [12,13]—challenges that would be amplified with multi-stage microreactor comprising numerous, successive DLD arrays within a single device. Previously, our group has demonstrated a multi-layer (or vertically stacked" DLD array manufactured *via* DLW-based 3D

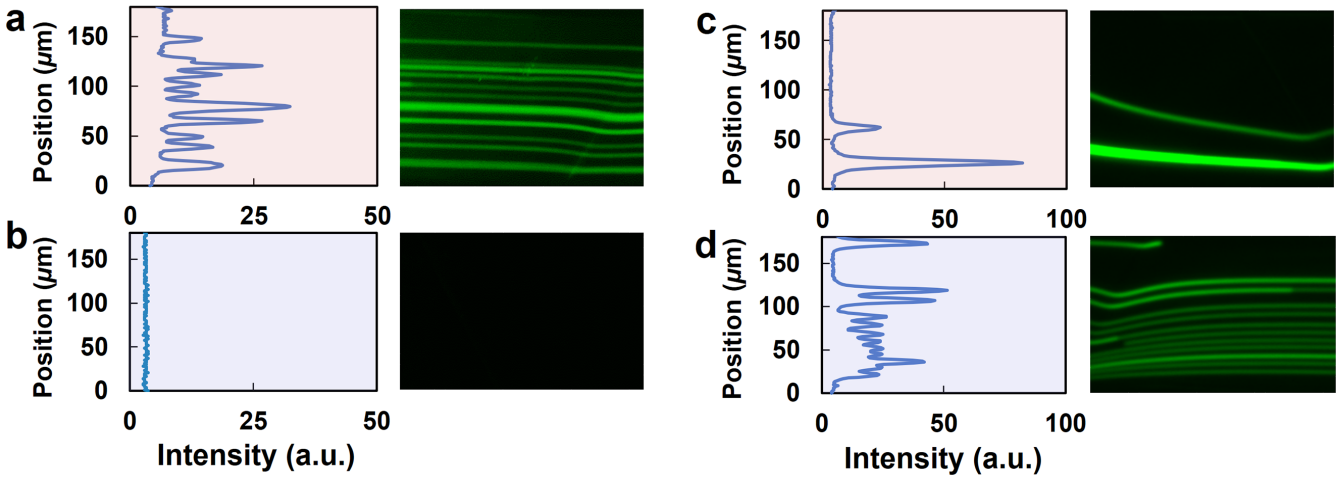

Figure 4: *Quantified fluorescence intensity results (left) and corresponding fluorescence micrographs (right) associated with the* ***(a,b)*** *inlets and* ***(c,d)*** *outlets for the* ***(a,c)*** *microparticle suspension fluid and the* ***(b,d)*** *surrogate 'reactant' fluid.*

printing that effectively sorted microparticles and nanoparticles based on size [18]. Future efforts should focus on investigating the potential of extending the vertically stacked DLD array strategy to incorporate the parallel flow transport concepts presented here, which could, in turn, provide novel means to enable multi-stage particle reactors that yield high throughput (while bypassing clogging failures) for wide-ranging applications in chemical and biological fields.

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