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Optical microassembly platform for constructing reconfigurable microenvironments for biomedical studies

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Abstract: Cellular development is highly influenced by the surrounding microenvironment. We propose user-reconfigurable microenvironments and bio-compatible scaffolds as an approach for understanding cellular development processes. We demonstrate a model platform for constructing versatile microenvironments by fabricating morphologically complex microstructures by two-photon polymerization (2PP) and then assembling these archetypal building blocks into various configurations using multiple, real-time configurable counterpropagating-beam (CB) traps. The demonstrated capacity for handling feature-rich microcomponents may be further developed into a generalized microassembly platform.

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1. Introduction

One of the central paradigms in biology is that structure and function are correlated at all levels of biological organization. Thus, it is not entirely surprising that, beyond biochemical signals, the topographic features of its microenvironment can exert a controlling influence on cellular behavior [1-3]. Contemporary advances in microfabrication technology have boosted systematic search for valuable insights on cell regulation effects based on micro- and nanoscopic features of the cellular environment (e.g. see [4,5]). Similarly, advances in micromanipulation may be exploited, as for instance in Ref. 3, where growing yeast cells were optically manipulated to show that confinement is a determinant of growth in a mixed yeast culture. The creative adaptation of contemporary technologies as investigative biomedical tools is a welcome development since, as expressed in Ref. 6, a multidisciplinary approach is highly necessary for bridging the gap towards fully understanding the underlying mechanisms. Such understanding will be a prerequisite for advancing biomedical solutions such as developing standard protocols for user-defined stem cell differentiation and tissue engineering for regenerative medicine.

Judging from scientific evidence on cellular response to topographical cues from its microenvironment, we conjecture that achieving reconfigurable microenvironments is a promising step towards unraveling the underlying physical mechanisms, or at the least improving operational knowledge, for controlling cellular behavior. In this work, we demonstrate an archetypal optical platform for constructing such reconfigurable microenvironments. While most previous work focused on the effects of substrate topography, we envision a three-dimensionally configurable environment. Specifically, we exploit two-photon photopolymerization as a microfabrication technique due to its capacity for sculpting model building blocks having complex three-dimensional morphologies with substructures down to nanometric scales [7-9]. It is worth noting that photopolymerizable materials with increased biocompatibility are actively being developed [10], which can be exploited in future biomedical experiments. These feature-rich building blocks can then be optically manipulated in a fluidic environment using multiple, real-time reconfigurable counterpropagating trapping beams to construct user-defined configurations or reconfigurable scaffolds. This approach complements the allied technique of directly fabricating 3D scaffolds by photopolymerization [11], which has been applied, very recently, to fabricate biodegradable structures [12]. Moreover, reconfigurable assembly has the added flexibility for utilizing building blocks made from different materials, which allows for selective chemical functionalization [13]. Reconfigurability enables user-defined "runtime adjustments" and its utility is appreciated

knowing how reconfigurable spatial light modulators carve their niche in relation to prefabricated masks.

Earlier demonstrations have shown optically assembled 3D architectures but these were limited to using either simple microspheres [14-16] or generally planar, nonspherical elements [17]. Furthermore, these assembled structures required constant laser illumination to prevent constituents from drifting apart. Thus, the structural components are held in place by optical forces in the piconewton regime. In the envisioned system, feature-rich microelements are imbibed with latching mechanisms to assemble robust structures that can be maintained even upon deactivating the trapping beams to minimize parasitic effects from laser irradiation. Smaller microelements can be anchored onto larger structures to suppress Brownian dynamics. We will show that our microassembly system, based on the BioPhotonics Workstation [18,19], exhibits the necessary degrees of freedom to tackle the exacting demands arising from the increased system complexity. Experiments show that the multiple optical traps achieve sufficient degree of translational and orientational control for maneuvering the microfabricated objects in 3D.

2. Design considerations and fabrication of microscale building blocks

A microfabrication system based on two-photon photopolymerization [20], was used to fabricate microcomponents through voxel-by-voxel solidification of an epoxy-based resin (SU8 , Michrochem, Newton, MA, USA) using tightly focused ultrashort pulses (Ti:sapphire laser, 100 fs, 80 MHz repetition rate, λ=796 nm). Setting the laser average power to 1.5 mW (focused by a 100× oil-immersion microscope objective) and using a scan speed of 8 μm/s, voxels can be solidified with minimum transverse and longitudinal dimensions of about 0.45 ± 0.1 μm and 0.8 ± 0.1 μm, respectively. In envisioned future biomedical experiments, the building blocks can be imbibed with nanometric features as needed for triggering cellular reactions and will include latching mechanisms for constructing robust microstructures while still allowing desired reconfiguration. In the present proof-of-concept demonstration, we fabricate two types of primitive microcomponents possessing complementary shape-features that enables joining them together to form hierarchical microstructures (see the scanning electron microscopy (SEM) images in Fig. 1). The microdumbbell is 13 μm long and contains identical spherical endings, 3.8 μm in diameter, connected by a cylindrical rod 2.2 μm in diameter. The dumbbells are designed to attach onto a complementary microblock (8 μm thickness and 17.5 μm side length). This is accomplished by fitting the dumbell's spherical endings onto circular holes (diameter = 5 μm) on the sides of the microblock.
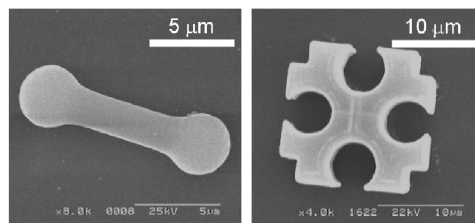

Fig. 1. Scanning electron microscope (SEM) images of 2PP-fabricated microstructures. The submicron lateral and axial resolution of resin solidification and the nanometer-precise 3D scanning paths enable 2PP to render the fine features of the microscale dumbbell (left) and its complementary primitive (right).

The dumbbell primitives serve a dual purpose in the present demonstration. From a biological perspective, a rod that is coupled to a microblock can mimic naturally occurring biological features like cilia that protrude from mammalian cells [21]. From an engineering perspective, the dumbbells serve as prototypical coupling shafts for joining microblocks into a desired configuration. In any case, assembling the complementary structures requires 3D micromanipulation since the dumbbell lobes cannot be inserted from the sides but must be

maneuvered from the top to achieve a latching effect. This microassembly was successfully carried out with a real-time reconfigurable array of counterpropagating-beam (CB) traps in the BioPhotonics Workstation, as described in the next section.

3. Reconfigurable microenvironment through parallel and interactive optical micromanipulation

A CW fiber laser beam (λ=1064 nm, IPG Photonics) was transformed into two matched sets of real-time user-adjustable multiple top-hat beams using spatial light modulation technology. The beam sets are coupled through facing microscope objectives (50×, NA = 0.55, Olympus) and enter from opposite sides of a sample chamber to work as counterpropagating beam traps for the fluid-borne microstructures (a small amount of surfactant prevents the particles from sticking together or to the substrate). Each counterpropagating beam (CB) trap consists of twin top-hat beams whose radii are matched to the structure to be trapped (e.g., ~4 μm radii for the dumbbell's spherical endings). Top-hat beams evolve as they propagate and in our usual implementation of CB traps the planes where the counterpropagating beams form top-hat profiles are separated by ~40 μm. Stable three-dimensional trapping is obtained within the region where the propagated beams overlap and axial manipulation is performed by varying the power ratio between the constituent beams of a CB trap [22]. For the present experiments, the power of each constituent beam is adjustable from 0 to ~3 mW and provides the desired particle control along the beam axis.

Multiple CB traps are simultaneously configured through a computer graphical user interface and form optical potential landscapes for controlled manipulation of microcomponents. By employing a tandem of two adjustable CB traps, each dumbbell microstructure can be optically controlled and manipulated with three translational degrees of freedom (DOF) for its center-of-mass (x, y, z) and two angular DOF for its long-axis orientation (θ, ϕ), as depicted in Fig. 2(a) and demonstrated by the snapshots of video recordings from optical trapping and micromanipulation experiments (see (Figs. 2(b)–2(g)) Stable axial position control of the dumbbell primitives was achieved over a 12 μm dynamic range. Steady trapping with an extensive range of tip-tilt adjustment of the dumbbell's long axis was done by applying varying power ratios to each lobe with programmed concurrent change in the transverse separation according to trigonometric constraints (e.g., see D and D_0 in Fig. 2(e)). Stable trapping in 3D is achieved within tip-tilt ranges of $\theta \in$ [0, 360°] and $\phi \in$ [–50°, 50°].
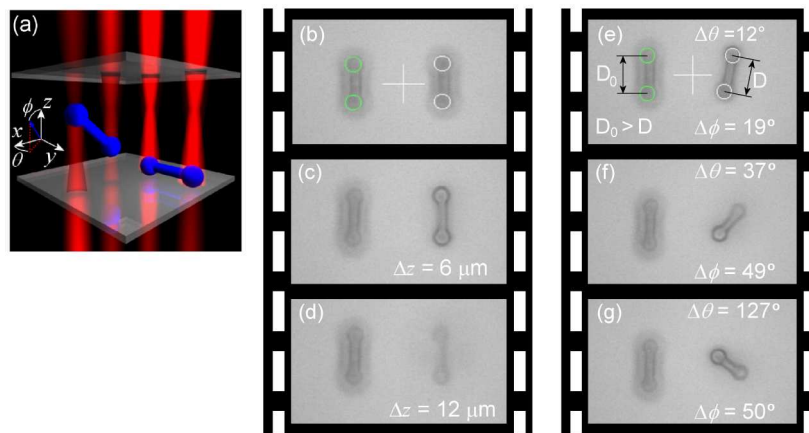

Fig. 2. Stable 3D optical manipulation of a microstructure. (a) Schematic of multiple counter-propagating-beam traps for controlled manipulation of dumbbell microstructures with five degrees of freedom (x, y, z, θ, and ϕ). (b)–(d): Snapshots of controlled axial displacement of a dumbbell microstructure at fixed azimuth and zenith orientations (Media 1, 2x speed). (e)–(g): Snapshots of tip-tilt control of a dumbbell microstructure (Media 2, 2x speed). A second set of optical traps holds another dumbbell in place for reference. Overlays show relevant quantities.

To demonstrate the optical microassembly of reconfigurable microenvironments, pairs and quadruplets of CB traps were used to manipulate dumbbell microstructures and their complementary primitives, respectively (Fig. 3(a)). First, a dumbbell is optically lifted to an axial position above the microblock (Fig. 3(b)). Transverse translation along the xy-plane is introduced until it can be brought down with one lobe fitting in a receptor hole. The procedure is then repeated to link the other end of the dumbbell to another separately controlled microblock (Fig. 3(c)). Once the assembly has been carried out, all existing CB traps can be grouped as one cluster with their lateral positions fixed relative to each other. As shown in Fig. 3(d), the cluster of traps can then be used to move and rotate the whole assembly in the xy-plane. Such functionality may prove useful for tasks in the assembly process when a partially assembled structure must be brought in proximity with and properly oriented with respect to another microcomponent, sub-assembly, or even a biological specimen during an experiment. Alternatively, the clustered CB traps can hold the assembled microstructure in place while either the sample stage is laterally scanned or a microfluidic flow is introduced to introduce more microcomponents into the optical trapping region. A large number of CB traps can be simultaneously created over an area of about 100 μm × 100 μm. Synthesis of multiple CB traps can be further scaled to a larger area since low-NA (air-immersion) objectives can be employed to project the beam array to the sample [16]. Thus, parallel assembly of several microsystems comprising of a few components is realizable. Furthermore, automated or closed-loop (video feedback) control may be employed to gather and assemble the microscopic building blocks using image-processing algorithms [17,23].
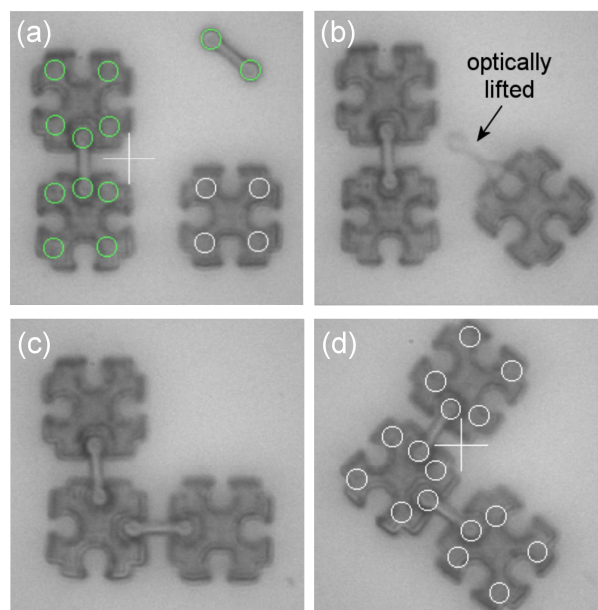

Fig. 3. Snapshots from acquired video illustrating optical microassembly of reconfigurable microenvironments using 2PP-fabricated components (Media 3, 3x speed). (a) A cluster of four CB traps is selected to rotate a microblock in 2D. (b) 3D optical manipulation of dumbbell microstructures with a pair of CB traps fitting spherical lobes to holes of complementary microblock. (c) Successful assembly of all the microcomponents. (d) Grouping all CB traps to collectively manipulate the entire assembly of microscale building blocks.

4. Summary and outlook

We have demonstrated an optical microassembly platform that uses multiple real-time adaptive 3D optical traps to construct reconfigurable microenvironments out of feature-rich microstructure primitives fabricated by multiphoton photopolymerization. For envisioned future experiments using building blocks synthesized out of biocompatible materials, we

believe that this system can help researchers investigate microenvironment-related biomedical questions such as regulatory mechanisms for cellular development or reconfigurable scaffolds for 3D tissue engineering. The facility for incorporating a CARS system into this platform [19], opens further possibilities for allied biochemical probing. Looking ahead, this scheme may be extended into a generalized microassembly platform for realizing a plurality of different microdevices and micromachines that can complement approaches based on self-assembly and mechanical micromanipulation [24]. Other technologies, such as photolithography and electron beam etching, may be used as complementary microfabrication processes for creating microscale components from a wider variety of materials.

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