Femtosecond Mathieu Beams for Rapid Controllable Fabrication of Complex Microcages and Application in Trapping Microobjects

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Supporting Information

ABSTRACT: Structured laser beam based microfabrication technology provides a rapid and flexible way to create some special microstructures. As an important member in the propagation of invariant structured optical fields, Mathieu beams (MBs) exhibit regular intensity distribution and diverse controllable parameters, which makes it extremely suitable for flexible fabrication of functional microstructures. In this study, MBs are generated by a phase-only spatial light modulator (SLM) and used for femtosecond laser two-photon polymerization (TPP) fabrication. Based on structured beams, a dynamic holographic processing method for controllable three-dimensional (3D) microcage fabrication has been presented. MBs with diverse intensity distributions are generated by controlling the phase factors imprinted on MBs with a SLM, including feature parity, ellipticity parameter q, and integer m. The focusing properties of MBs in a high numerical aperture laser microfabrication system are theoretically and experimentally investigated. On this basis, complex two-dimensional microstructures and functional 3D microcages are rapidly and flexibly fabricated by the controllable patterned focus, which enhances the fabrication speed by 2 orders of magnitude compared with conventional single-point TPP. The fabricated microcages act as a nontrivial tool for trapping and sorting microparticles with different sizes. Finally, culturing of budding yeasts is investigated with these microcages, which demonstrates its application as 3D cell culture scaffolds.

KEYWORDS: two-photon polymerization, Mathieu beams, polymer microcages, dynamic holographic processing, microobject trapping

Trapping and holding microobjects has been a hot research focus in recent years, which has important applications in biomedical research and fundamental studies of cell behavior. Optical tweezers,1–3 a simple and robust implementation for microobject manipulation, have been successfully used to manipulate microobjects from several micrometers to tens of nanometers. However, an optical tweezer is usually generated by a tightly focused laser beam, which is harmful to living samples or chemical reactions. Therefore, to address these issues, some specifically designed microstructures were fabricated for microobject manipulation.4,5 As an example, hierarchical helical arrays realized by capillary force assembly of micropillar arrays,5 which was fabricated by the two-step soft transfer of a silicon template prepared by UV lithography and plasma etching, were used to trap many particles rapidly and simultaneously. Miniaturized self-folding grippers which are actuated by differential residual stress,6 temperature,7,8 magnetic force,9 and chemicals10,11 can trap microparticles inside “cage-like” spaces. Moreover, corner
lithography, which is a wafer-scale nanopatterning technique involving conformal deposition and selective isotropic thinning, can form arrays of nanowire pyramids in sharp concave corners for microbeads and cell trapping. Although a variety of complex microstructures have been achieved, many of them have distinct drawbacks of complicated fabrication processes or dependence on expensive apparatus and low controllability. Therefore, it is still in high demand to develop a simple, rapid, and controllable method to fabricate desired micro/nanostructures for manipulating microobjects.

The two-photon polymerization (TPP) technique is extensively studied for fabricating complex functional 3D micro/nanodevices, owing to its simple operation, sub-100 nm resolution, and intrinsic three-dimensional (3D) processing ability. However, the wide application of TPP is restricted by the low fabrication speed due to the single-point scanning strategy. In order to improve the efficiency, typical methods, such as multifoci parallel fabrication by micro lens arrays, diffractive optical elements, or multibeam interference, are developed. Nevertheless, the disadvantage of these methods is that the positions of the foci are fixed by the optical elements. Moreover, these methods are only suitable for fabricating microstructures with the same morphology. It is a much more flexible way to fabricate complicated microstructures by using a SLM. Until now, multifocal fabrication of functional microstructures has been performed by adopting SLM, such as microoptics and microfluidic structures. Besides the multifoci array, structured beams are capable of fabricating specific microstructures with high efficiency. Mathieu beams, as a kind of special structured beams, have two interesting features: "diffraction-free", that is, preserving their shape while propagating, and "self-healing", that is, recovering to its original shape even if part of the beam is destroyed by a small obstacle. Due to these specific features, MBs have already been used for 3D optical trapping and micromanipulation. However, adopting MBs in femtosecond laser TPP processing for rapid fabrication of functional 3D structures has not been reported so far. The focusing properties of MBs under high numerical aperture (NA) and the impact of laser beam parameters on fabricated micro/nanostructures have not been quantitatively investigated.

In this work, we present a dynamic holographic processing method to fabricate 3D microcages. First, MBs are generated by using a liquid-crystal phase-only SLM without any other optical elements. The focusing properties of MBs in a high NA laser microfabrication system are investigated by Debye vectorial diffraction theory. The relationship between intensity distribution of focused MBs and phase factors imprinted on MBs by SLM, including feature parity, ellipticity parameter $q$, and integer $m$, is theoretically and experimentally studied. On this basis, a simple method to fabricate 3D microcages by precisely scanning the MBs and Bessel beams (BBs) along the $Z$ direction in the photore sist is proposed and experimentally verified. This strategy exhibits great flexibility because the patterned focus can be dynamically controlled. The fabrication speed can be increased by 2 orders of magnitude compared with conventional single-point direct laser writing. Finally, effective trapping/sorting (SiO$_2$ particles) and trapping/culturing (in vivo culturing of yeast) using the resultant 3D microcages are demonstrated, leading to applications in microfluidics and biomedical study.

RESULTS AND DISCUSSION

Mathieu Beam Generation. The generation of MBs is accomplished by displaying the phase of MBs on the SLM, behind which nondiffraction MBs are reconstructed on-axis. The phase masks we imprinted on SLM for even, odd, and helical MB generation can be expressed, respectively, as

$$q^c_m = \text{Im}(\log M^c_m(\eta, \xi, q))$$

$$= \text{Im}(\log(C_m f_m(\xi, q) s_m(\eta, q))), \ m = 0, 1, 2, 3...$$

$$q^o_m = \text{Im}(\log M^o_m(\eta, \xi, q))$$

$$= \text{Im}(\log(S_m f_m(\eta, q) s_m(\eta, q))), \ m = 1, 2, 3...$$

$$q^{HLM}_m = \text{Im}(\log M^{HLM}_m(\eta, \xi, q))$$

$$= \text{Im}(\log(M^c_m(\eta, \xi, q) \pm M^o_m(\eta, \xi, q))), \ m = 1, 2, 3...$$

Here, $f_m(\xi, q)$ and $s_m(\eta, q)$ are the even and odd modified Mathieu functions of order $m$, and $c_m(\eta, q)$ and $s_m(\eta, q)$ are the even and odd ordinary Mathieu functions of order $m$, and $C_m$ and $S_m$ are constant. The ellipticity parameter is defined as $q = f^2 k^2 / 4$, where $k$ is the transverse spatial frequency and $f$ is the semifocal distance. Elliptical coordinates $(\xi, \eta)$ are given by Cartesian coordinates $(x, y)$ of SLM pixels through the transformation $x = f \cosh \xi \cos \eta; \ y = f \sinh \xi \sin \eta$.

Figure 1a illustrates the experimental setup of TPP fabrication using MBs. The incident Gaussian beam is modulated by a computer-generated hologram (CGH) displayed on the SLM. All the CGHs used in the experiment need to be pretreated, as shown in Figure 1b. Handling CGHs in this way serves two purposes. First, due to the pixilation effect of the SLM, blurred grating (BG) phase can separate the zero-order light away from the other modulated light. Thus, with an iris diaphragm, the effect of zero-order light on the fabricated microstructures can be effectively reduced. Second, because the cross section of Gaussian light incidents on the SLM is circular, the CGHs are also tailored to a circle to better match the incident light. The phase distribution of BB is expressed as $2\pi x / \Delta$, in which $\Delta$ represents the period of the BG (15 pixels), and the pixel pitch of SLM is 8 $\mu$m. So the final phase distribution of the CGH loaded on SLM can be expressed as $\Phi(x, y) = \text{mod}(\phi_m(x, y) + \phi_{BG}(x, y), 2\pi)$, where “mod” is the remainder operation, $\phi_m(x, y)$ is the Mathieu beam phase, and $\phi_{BG}(x, y)$ is the BG phase.

The intensity distribution of MBs along the propagation axis is calculated by the Fresnel diffraction theory as the paraxial approximation condition is satisfied. The intensity profiles in the transverse direction, at distances of 0.4, 0.7, 1.0, 1.3, and 1.6 $\mu$m from the SLM, are calculated and experimentally measured (Figure S1). It can be seen that the intensity profiles change little both in theoretical prediction and in experimental measurement, which demonstrates the propagation invariant property of MBs.

Further, simulation of the transverse intensity distribution of the reconstructed optical field at different planes is shown in Figure 1c. The intensity distribution from plane 1 to plane 4 corresponds to the intensity distribution at the front plane of Lens1 (600 mm), focus of Lens1, back of Lens2 (200 mm), and the entrance pupil of the objective, respectively. It is found that the intensity distributions exhibit a main multifoci ring
containing most of the energy and additional surrounding pattern containing much lower energy. An iris was placed at the Fourier plane of Lens1 to filter out unwanted orders and let MBs pass through, as shown in Figure S2.

**Focusing Properties under High Numerical Aperture Objective Lens with Different Focal Length Ratio Lens Group in a 4f System.** It is well-known that the diffraction behavior of light tightly focused by a high NA objective is different from the scalar diffraction. In order to facilitate the visualization of the field distribution at the focal region, we used Debye vectorial diffraction theory for optical field simulation. The intensity distribution in the focal region can be rewritten as

\[
E_i(x_1, y_1, z_1) = \frac{IC}{\lambda} \int_0^a \int_0^{2\pi} \sin \theta_1 \cos \theta_1 P(\theta_1, \phi_1) \times \\
\exp[i k n_0 (z_1 \cos \theta_1 + x_1 \sin \theta_1 \cos \phi_1) + y_1 \sin \theta_1 \sin \phi_1] d\theta_1 d\phi_1
\]

(4)

where C is a constant; \(\lambda\) is the wavelength of incident light; \(n_0\) is the refractive index of the immersion medium; \(a\) is the maximum focusing angle of the objective lens and can be calculated by \(a = \arcsin(r \text{NA}/R_{n0})\); \(r\) is the off-axis coordinate of the incident wave; \(R\) is the object lens pupil radius; \(\theta_1\) is the focusing angle of the objective lens, and \(\phi_1\) is the azimuthal angle of object plane. \(P(\theta_1, \phi_1)\) is the polarization state of the EM field in the focal region, which can be expressed as

\[
P(\theta_1, \phi_1) = [1 + (\cos \theta_1 - 1) \cos^2 \phi_1] i \\
+ [(\cos \theta_1 - 1) \cos \phi_1 \sin \phi_1] j - (\sin \theta_1 \cos \phi_1) k
\]

(5)

for incidence with linear polarization in the X direction.

In the TPP fabrication system, many factors will affect the light field distribution, such as the focal length ratio of the lens in the 4f system. To show the effect of focal length ratio on the focused light under high NA objective lens, theoretical simulations and experiments are conducted. According to eqs 4 and 5, taking the even-MBs, for example, simulated optical intensity distribution with the focal length ratio of lenses \(L_1\) and \(L_2\) changed from 1:1 to 5:1, as shown in Figure 2. As the focal length ratio of the lenses \(L_1\) and \(L_2\) becomes larger, the diameter of the light field entering the pupil is reduced (Figure 2a) and the diameter of the focused light field (Figure 2b) under the objective lens becomes larger (marked in a white line) and the Z direction is stretched (Figure 2c). The reason is that the numerical aperture of the objective lens is not fully utilized due to the smaller diameter of the incident beam. The ratios of 1:1 and 3:1 were experimentally measured (shown in Figures S3 and S4), which is in agreement with the theoretical prediction. It is found from the experimental light field in the X–Y plane along different Z positions that a discrete intensity profile becomes a single-ring structure gradually. With this factor, it is possible to obtain focused light with different diameters and different Z direction lengths by changing the ratio of the lens groups.

**Optical Intensity Distributions and Corresponding Microstructures Fabricated by Mathieu Beams with Different Feature Parity and Integer \(m\).** Mathieu beams are characterized by three parameters: the feature parity, the ellipticity parameter \(q\), and the integer \(m\), which is termed topological charge. We systematically studied the effects of these parameters on the intensity profile of MBs and the final fabricated microstructures. On the basis of eqs 1–3, it can be seen that only in the case of even-MBs, the integer \(m\) begins from 0 and the remaining two cases are from 1. Figure 3a is a series of CGHs with different parameters for even-MBs, in which the bright and dark gray values correspond to a phase of 0 and \(\pi\), respectively. These CGHs have a similar feature parity (even) and ellipticity parameter \(q\) of 5 without the loss of generality. The integer \(m\) changes from 0 to 6 successively. From the simulated results in Figure 3b, it can be seen that even-MBs show multifoci arranged in a ring manner at the focal plane. Around the focus ring, there is still a little stray light with weak intensity. Apparently, the number of focus \(N_m\) in the ring is directly related to the integer \(m\), which can be expressed as \(N_m = (m + 1) \times 2 (m = 0, 1, 2, 3)\) and \(N_m = m \times 2 (m \geq 4)\). The diameters of the rings remain unchanged. The measured results are consistent with the simulation (Figure 3c). In the experiment, a focused optical field is projected into a \(-8 \mu m\) thick SZ2080 photosensitive resist, and complex 2D multifoci patterns can be fabricated by single exposure without single-point scanning. In order to ensure sufficient TPP all through the polymer, a longer focal length in the Z direction is needed, then the focal length ratio of lenses \(L_1\) and \(L_2\) is selected as 3:1. All the microstructures are fabricated with a laser power of 110 mW, which is measured in front of the objective, and an exposure time of 100 ms (Figure 3d). Similar results for odd-MBs are shown in Figure 3e–h. Likewise, the
measured intensity profile and fabricated microstructure agree well with the simulation. It is worth noting that the relationship between the number of focus $N_m$ and the integer $m$ is different from the even-MBs, which can be expressed as $N_m = m \times 2$. The results for helical MBs are shown in Figure S5, and the relationship between the number of focus $N_{mH}$ and the integer $m$ is the same as the odd ones.

We can find that the multifoci microstructures are not perfectly homogeneously polymerized. This is caused by three aspects. First, the optical system in the experiment was not perfect. For example, all lenses in the optical system are hardly 100% completely coaxial, and it is difficult to completely guarantee the laser beam focus perfectly vertically to the sample after passing through the microscope system. These nonideal hardware conditions may bring errors to the fabrication. Second, there is a refractive index mismatch between different materials behind the objective, which will bring aberrations. Finally, linear polarization incidences also lead to an inhomogeneous intensity distribution after high NA focusing. In order to enhance the resolution and uniformity of fabricated final structures, the point spread function of the patterned focus can be optimized by compensating the aberration of high NA fabrication systems.

Optical Intensity Distributions and Corresponding Microstructures Fabricated by Mathieu Beams with Different Ellipticity Parameter $q$. From previous results, it is obvious that when the ellipticity parameter $q$ is constant, the diameters of the microring structures are constant at different integers for all even, odd, and helical MBs. Here, we take the even-MBs with integer 6 as an example to analyze the effect of $q$ on the diameter of the fabricated microstructure, without the loss of generality. Figure 4a is a series of CGHs for the generation of even-MBs with the same integer $m = 6$ and $q = 6$, without the loss of generality. Figure 4a is a series of CGHs for the generation of even-MBs with the same integer $m = 6$ and a set of different ellipticity parameters. From the simulated results in Figure 4b, it can be seen that the diameter of the intensity pattern at the focal plane increases with the ellipticity parameter changing from $q = 1$ to $q = 20$. The measured diameter change is consistent with theoretical simulation. Meanwhile, the smaller the ellipticity parameter became, the more evenly spaced the multifoci is. In Figure 4d, all of the microstructures are fabricated by a single exposure with the intensity profile in Figure 4c in a ∼8 μm thickness SZ2080 photoresist sample. When the ellipticity parameter is $q = 1$, the single exposure time is 80 ms with a laser power of 100 mW. When the ellipticity parameter is $q = 15$, the single exposure time is 400 ms and a laser power of 140 mW is needed. As the diameter of the microstructure increases, the needed single exposure time and laser power also increase. All processing parameters are displayed in Table 1 in the Supporting Information. As mentioned in the above section, the focal length ratio of lenses L1 and L2 is selected as 3:1 to ensure that TPP occurs all through the polymer. For the precise control of microstructure diameter, a quantitative study between the diameter and ellipticity parameter $q$ is conducted. The red line in Figure 4e represents the simulated results. When ellipticity parameter $q$ changes from 1 to 20, the diameter of microstructures varies from 4.3 to 21 μm. The black line represents the average of five measured microstructures under the same experimental conditions. Correspondingly, the average diameters of microstructures vary from 4.1 to 20.2 μm. In the simulation, the diameters are calculated according to the full width at half-height of the intensity distribution.
should be noted that a wider range of diameters of the microstructures could be obtained when an appropriate beam reducer and low magnification objective are used.

The deviation between measured and calculated diameters may come from two aspects. The first and the most important reason is the shrinkage of the photoresist, especially when the laser exposure dosage is close to the photoresist polymerization threshold. The second reason is that the simulation is based on a series of ideal experimental conditions. For example, the incident light we supposed is a uniform standard Gaussian beam, and the optical system is idealized. Actually, errors will be caused by the pixelization of holograms, the high NA objective system, and the nonideality of the optical system.

With this MB-based TPP processing, a complex 2D microstructure can be easily fabricated combined with the movement of the sample anchored to the piezoelectric platform. Figure 4 f,g and Figure S5 are the SEM and fluorescence images of patterned microring array. The processing parameters for each microring are the same as in Figure 4d. Dynamic Holographic Processing of Various Controllable 3D Microcages. Based on systematic study on TPP with MBs, a method for rapid fabrication of 3D microcages by scanning patterned focus along the Z direction is proposed. Different from the conventional TPP process by single-point scanning, this method can greatly reduce the processing time. Furthermore, by changing the tailored holograms during the scanning of patterned focus with evenly spaced, microcages with variable cross section could be successfully produced. With this dynamic holographic processing method (DHP), we can easily and rapidly fabricate different kinds of microcages. Figure 5a is the schematic illustration of the DHP method. Different layers are processed by MBs and BBs. Taking single-layer microcages as an example, the stretching speed of the platform is 50 μm/s and the designed height of the bottom pillars in the microcages is 15 μm. So the fabrication time is 0.3 s, which means the hologram (Mathieu beam) is displayed for 0.3 s. Meanwhile, the designed height of the junction in the microcages is 3 μm, making the fabrication time 0.06 s, which means the hologram (Bessel beam) is displayed for 0.06 s. Therefore, with the integrated control software, the dynamic holographic processing has been realized. We define the exposure time as the fabrication time. In the experiment, the thickness of photoresist is ~500 μm, which ensures that there is enough space to process the 3D microcages. For precise 3D microfabrication, a higher Z direction resolution is required, and the focal length ratio of lenses L1 and L2 is selected as 1:1. Figure 5b is the SEM image of single-layer microcages with eight pillars at the bottom. The microcages have ~18 μm height and ~10.5 μm diameter. The fabrication time for one
single-layer microcage is 0.36 s, whereas it increases to 54 s for conventional single-point scanning (Figure 5c), which means that the fabrication time can be reduced by 2 orders of magnitude. Figure 5d is the SEM image of two-layer microcages with eight pillars at the bottom layer and six pillars at the top layer. The microcages have \( \sim 36 \mu m \) height and \( \sim 10.5 \mu m \) diameter. The stretching speed for fabrication is 50 \( \mu m/s \), whereas the used power is 90 mW. Similarly, the fabrication time can reduce from 102 to 1.12 s (Figure 5e) compared with single-point TPP processing in theory. Figure 5f is the SEM image of three-layer microcages with eight pillars at the bottom, six pillars at the middle, and four pillars at top. The microcages are \( \sim 54 \mu m \) in height, and the diameters are the same as in Figure 5b,d. Compared to the single-point scanning method, the fabrication time is 100 times less.

Because the scanning strategy is moving the sample along the Z direction, the height of the microcages could be fabricated as long as the working distance of the oil immersion lens. Therefore, this processing method can fabricate high aspect ratio microcages (Figure 5h). These microcages have \( \sim 90 \mu m \) height, and the aspect ratio is 9:1 approximately. Further, the location of microcages can be well designed; for example, a "USTC" pattern was designed by microcages with different heights (Figure 5i). It is challenging for other techniques to fabricate these 3D microcages, and thus our approach shows the distinct advantages in terms of 3D and customization capabilities.

### 3D Microcages for Trapping Microobjects

The ability to manipulate microobjects is highly desirable in biological devices and chemical analysis and has drawn great attention. Although entirely new perspectives are provided by the complex patterning of light fields in advanced optical tweezer micromanipulation, substantial 3D microstructures are still desired to be fabricated so that the energy beam does not cause harm to living samples or chemical reactions. In this work, we demonstrate the feasibility of 3D microcages fabricated by DHP for trapping particles, as illustrated in Figure 6a.

A deionized water solution containing 5 \( \mu m \) diameter SiO\(_2\) particles is dropped on the sample, which is fabricated on a glass slide. Part of particles are trapped in the microcages by gravity and hydrodynamic forces. Then, the sample is cleaned by deionized water and shaken slightly to flush the nontrapped particles away. The trapped particles are confined in the microcages (Figures 6b–d). Trapping efficiency is an important factor to evaluate the trapping capability. We define the trapping efficiency as the ratio of the microcages that trap SiO\(_2\) particles with success to the total number of microcages. In our experiments, the trapping efficiency can reach more than \( \sim 90\% \) (Figure 6a and Figure S7) when the number of repeating cycles exceeds 5. After we repeated the trapping experiments for several times (>5), the microcages can trap microparticles in every experiment and have no obvious deformation.
In recent years, microobject sorting has been important to enrich or purify biosamples into well-defined groups for plenty of applications. A variety of microfluidic sorting methods based on 2D substrates have been developed to separate microparticles based on size. Here, we show that our 3D microcages can separate particles of different diameters by changing the number of pillars at the bottom, similar to the process in Figure 6a. A deionized water solution containing different SiO₂ particles with various diameters (e.g., 2, 3, 4, 5, 6, and 7 μm) is dropped on the samples fabricated on the slide. There are two types of microcages. One is single-layer microcages with four pillars at the bottom (Figure 6e), and the other is single-layer microcages with eight pillars at the bottom (Figure 6h). Due to different number of pillars, the distance between the pillars is different. For four pillars microcages, small particles (2 and 3 μm) will be washed out from the gap (∼4.3 μm) between two adjacent pillars, while large particles (5 and 6 μm) remain inside the microcages (Figure 6f,g). In contrast, both small and large particles remain inside the microcages with eight pillars (Figure 6i,j), due to smaller pillar gap (∼1.2 μm). Therefore, particle sorting is achieved by these microcages with different shapes.

In general, the growth environment of cells in vivo is mainly 3D microenvironments, in which cell behaviors are different from that in 2D substrates. Here, the microcages can act as functional 3D cell culture scaffolds for cell trapping and culturing. As shown in Figure 7, yeast cells (Angel Yeast Co., Ltd.) are trapped and then subsequently cultured in liquid culture medium. The trapping of yeast is realized by dropping a few drops of high concentration yeast culture medium on the microcages. A part of the yeast are trapped in the microcages by gravity and hydrodynamic forces, after which the sample is cleaned by the culture medium. Finally, the growth and dividing process of trapped yeast is observed in situ with a bright-field microscope for 3 h in 20 mL of culture medium.

The 3 h culturing of yeast in the microcage can be divided into two steps. The first step is the growth of the primary generation of yeast (Figure 7a–c). The second step is the budding process and the growth of the first generation of yeast (Figure 7d–f). The dependence of the diameter of the yeast on culture time is shown in Figure 7g. With the increasing of culture time, the diameter of the primary generation of yeast increases obviously in the first step (blue area) but enlarges little when the budding process starts (gray area). After 0.5 h of culture, the first generation of yeast grows to a normal volume. The SEM images of the 3D microcage with the cultured yeast are shown in Figure 7h. This method provides a valid solution for observing cellular behaviors in the 3D microenvironment.

CONCLUSION

In summary, a rapid and flexible 3D microcage fabrication method based on dynamic holographic processing is developed. The focusing properties of MBs generated by phase modulation are theoretically and experimentally investigated. The 2D circle microstructures with multifoci are fabricated by single exposure of focused MBs. Moreover, 3D microcages with various geometries are fabricated by dynamically controlling the different patterned focuses. Compared with the traditional single-point scanning direct laser writing, the fabrication speed can be increased by 2 orders

Figure 5. Various kinds of 3D microcage fabrication with the dynamic holographic processing technique. (a) Illustration of the fabrication process of a three-layer microcage. Different layers are processed by MBs and BBs. The scanning speed is 50 μm/s, whereas the used power is 90 mW. (b,d,f) SEM images of different kinds of microcages. (c,d,f) Quantification of single structure processing time. The black column represents dynamic holographic femtosecond laser processing, and the red column represents single-point scanning. (h) SEM image for high aspect ratio microcages (height: ∼90 μm). (i) 3D microcage array with two different heights distributed in the “USTC” pattern. All scale bars are 10 μm.
of magnitude. These 3D microcages are demonstrated to have fine ability in trapping SiO₂ particles with a high trapping ratio and sorting microparticles with different shapes. Finally, the 3D microcages are used as cell culture scaffolds for the observation of growth and budding of yeast. We believe these controllable 3D microcages can act as a capture–hold–analyze system for a broad applications such as microfluidics and biological research.

METHODS

Numerical Simulation of the Intensity Distribution in the Focal Region under a High NA Objective. It is crucial to investigate the focusing properties of the MBs. Because the paraxial approximation does not consider the vectorial nature, in this work, we use Debye vectorial diffraction theory, which describes the depolarization effect of a high NA object by calculating the three orthogonal field components \(E_x\), \(E_y\), and \(E_z\).

Hologram Generation. In this work, we use Matlab software to generate and compute holograms (BG’s hologram and Mathieu beam’s hologram). All the holograms are designed to be matrices with the same dimension (1080 × 1080 in our experiments) corresponding to the modulated liquid crystal panel pixels of SLM. Every element value in the matrices is the phase to be modulated. As is known, the incident wavefront can be expressed as \(E(x,y) = A(x,y)e^{i\phi(x,y)}\). When it propagate through a pure phase plate \(\phi(x,y) = \phi(x,y)\), the modulated output beam can be expressed as \(E_{\text{out}}(x,y) = E(x,y) \times \phi(x,y) = A(x,y)e^{i\phi(x,y) + \phi(x,y)}\). In our experiment, we can modulate the incident Gaussian beam by the Mathieu beam’s hologram phase \(\phi_m(x,y)\) and the BG’s hologram phase \(\phi_{BG}(x,y)\), so we just need to add two matrices together numerically. The final phase distribution should be loaded on the SLM is \(\Phi(x,y) = \phi_m(x,y) + \phi_{BG}(x,y, 2\pi)\), where “mod” is a reminder operation.

Materials and Equipment. A commercially available zirconium–silicon hybrid sol–gel material doped with 4,4’-bis(diethylamino)benzophenone photoinitiator at 1 wt % (SZ2080, IESLFORTH) was used for photopolymerization in our experiment. Before microstructure processing, the SZ2080 was placed on a thermal platform set to be 100 °C for 1 h to evaporate the solvent. The femtosecond laser source is a mode-locked Ti:sapphire ultrafast oscillator (Coherent, Chameleon Vision-S) with central wavelength at 800 nm, pulse duration of 75 fs, and repetition rate at 80 MHz. The laser power is modulated with a half-wave plate and a Glan laser beam splitter. After expansion, the laser beam illuminates a phase-only SLM (Pluto NIRII, Holoeye, 1920 × 1080 pixels, 256 gray levels, and pixel pitch of 8 μm). The modulated beam is relayed to a 4f system and focused by a high NA object lens (60×, NA = 1.35, Olympus) into the photoresist, which is mounted on a piezoelectric platform (ES545, from Physik Instrumente GmbH & Co. KG, Germany) with nanometer resolution and a 200 μm × 200 μm × 200 μm moving range. After

Figure 6. Microparticle trapping and sorting by microcages with different pillar numbers. (a) Schematic procedure for the trapping of SiO₂ particles. Deionized water solution containing 5 μm diameter SiO₂ particles is dropped on the sample. Then, the sample is put in a Petri dish with deionized water and shaken slightly to flush away free particles. At last, almost all microcages are filled with particles after repeating this process several times. (b) SEM image of the sample after trapping SiO₂ particles. (c) Tilted view (45°). (d) Magnified view. (e,h) Schematic diagram for separating particles of different diameters using two different kinds of microcages. (f,g,i,j) SEM images of the separating results with top view and tilted view (45°). It is obvious that the microcages with four pillars (gap: ∼4.3 μm) at the bottom can only trap large diameter particles (5 and 6 μm) and small particles (2 and 3 μm) are washed out from the gap (∼4.3 μm) between two adjacent pillars, whereas the microcages with eight pillars (gap: ∼1.2 μm) at the bottom can trap particles with different diameters. All scale bars are 10 μm.
polymerization, the sample was developed in 1-propanol for 1 h until all of the unpolymerized parts were washed away.

Manipulation of Microparticles. SiO₂ microparticles with different diameters synthesized by Wakely Scientific Corp. Inc. were mixed in deionized water solution with a concentration of 10⁻³ g mL⁻¹. In order to make the SiO₂ particles evenly distributed in the deionized water solution, the container was shaken gently. The sample was horizontally placed on the stage of an optical microscope (Leica DMI3000B). The solution was dropped on the sample by a pipet, and most particles sank to the bottom after 10 min. In this process, a part of particles were trapped within the microcages. Then, the sample with trapped particles was transferred to a Petri dish with deionized water to wash untrapped particles away. Finally, the sample was mounted on the stage for a few minutes for the evaporation of the rest of the deionized water. This process is repeated for several times in order to achieve a high trapping ratio.

Yeast Culture. A kind of Saccharomyces cerevisiae (Angel Yeast Co., Ltd.) was used in the culture experiment. The liquid culture medium was produced by sterilizing the mixture of solid granular Sabouraud’s glucose broth medium (10.0 g L⁻¹ peptone, 40 g L⁻¹ glucose, pH 5.6 ± 0.2 at 25 °C, QingDao Hope Biotechnology CO., Ltd.) and distilled water with a concentration of 50 g L⁻¹ at 120 °C for 20 min. The high concentration yeast medium was produced by dissolving 0.1 g of dry yeast uniformly into 15 mL of liquid culture medium.

Imaging Characterization. The SEM images were taken with a secondary electron scanning electron microscope (ZEISS EVO18) operated at an accelerating voltage of 10 keV, after the samples were sputter coated with gold. The gold vaporization time is 80 s, and the gold thickness is ~10 nm.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.9b00893.

More details about numerical simulation, experimental results, and discussions (PDF)

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