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Microstructures Fabricated by Two-Photon Polymerization and Their Remote Manipulation Techniques: Towards 3D Printing of Micromachines

*Yang Lin and Jie Xu**

Mechanical and Industrial Engineering, University of Illinois at Chicago, Chicago, Illinois 60607, USA

Corresponding author: Jie Xu (jiexu@uic.edu)

Abstract

As a promising microfabrication method, two-photon polymerization (TPP) has received rapid development for various applications: chemistry, biology, pharmaceuticals, microfluidics and so forth. In particular, it has received intensive attention for applications of micromachines in recent years, including those require remote manipulation. Different manipulating techniques such as magnetic, optic and acoustic manipulation have been realized on TPP-fabricated microstructures, demonstrating the great potential for further development of micromachines. Nonetheless, most of the work are still at early stages, only proofing conceptual ideas. For instance, magnetically driven microswimmers have only been investigated in artificial experimental environments, and it is still challenging to tackle complex *in vivo* conditions. Although a long journey is still ahead for using these micromachines in daily life, it is predictable that the combination of two-photon polymerization associated with remotely driven techniques will benefit various fields.

Keywords: two-photon polymerization, magnetic, optic, acoustic, micromachines, microstructures

1. Introduction

Micromachines have caught ever-increasing attentions due to advantageous abilities manifested in micro-scale applications, which otherwise are difficult to achieve by macro counterparts. They have proven values in a variety of applications including electronics,[1] microfluidics,[2] biosensors,[3] robotics,[4] diseases diagnosis,[5] wireless communication,[6] etc. Nevertheless, in contrast to macro devices, precise manipulation of micromachines is still a critical issue especially for those used in harsh environments and enclosed situations such as *in vivo* organisms.[7] Built-in electrical circuits and peripheral wirings also hinder the developments in further miniaturization. Thereby, various driving methods have been studied and exploited over the past decades to address these problems, including magnetic force,[8] optical tweezer,[9] acoustic wave,[10] electrostatic force,[11] hydraulic force,[12] and the use of stimuli-responsive materials.[13] Among these methods, magnetic force, optical tweezer and acoustic wave have surpassed others due to noncontact and noninvasive manipulation, especially for biomedical applications. As an example, degradable microswimmers fabricated from a novel magnetic hydrogel have been developed recently.[14] Magnetic force was used to manipulate untethered microswimmers precisely based on the well-known cork-screw motion. Similarly, optical force has also been utilized.[15] To name a few, Galajda and Ormos have created a miniaturized rotor via two-photon polymerization and optical tweezer was used to trap and induce rotation of the rotor.[16] Kelemen and coworkers directly used the optical force from an integrated waveguide to manipulate the microrotor.[17]

Nowadays, great strides have been made to develop simple but reliable fabrication techniques for microstructures and micromachines, including UV nanoimprinting lithography,[18] chemical vapor deposition,[19] soft lithography,[20] two-photon polymerization (TPP),[17] etc. Among these techniques, TPP has superior merits when it comes to the fabrication of structures that demand complex spatial features with sub-diffraction-limited resolution. Additionally, movable components can be simply obtained without using sacrificial layers.[12] The remaining thin layer of monomers at the exterior surface of the fabricated structure also provides the opportunities for future chemical modifications. Last but not least, doped photoresists with additional properties further enable creative applications for manipulation of micromachines or their components.

Based on the hypothesis that two photons can be absorbed simultaneously as long as their total amount of the energies matches the energy gap between two electronic states, TPP was proposed in 1931 by Göppert-Mayer.[21] But the realistic experimental achievement was achieved after the invention of laser, which enables the coherent light with higher intensity.[22] Along with burgeoning development of stereolithography, the first three-dimensional (3D) structure created by TPP was published in 1997 by Maruo and colleagues.[23] From then on, TPP has received rapid development, and several excellent reviews and books have already been published,[18, 24] In this review, essential mechanism behind TPP and relevant photosensitive materials will be introduced first, followed by discussion regarding common treatments that make remote manipulation come true. Afterwards, applications with respect to various types of remotely driven techniques will be summarized and elucidated. Finally, conclusions will be drawn, and future prospects will be provided.

2. Mechanism and photosensitive materials for two-photon polymerization

As a type of microstereolithography (micro-SLA), TPP is different from traditional microfabrication techniques operated in a clean room. Owing to its voxel by voxel fashion, extra operations such as chemical or plasma etching, and mask involved ultraviolet (UV) exposure are dispensable, thus facilitating the process of fabrication especially for prototyping designs with irregular features.

Up until now, the configuration in modern machines for TPP does not differ too much from that of the first prototyping machine invented by Maruo and colleagues. Specifically, a mode-locked Ti:sapphire oscillator is used to generate consistent light with wavelength around 780nm, associated with a repetition rate of 80MHz. Afterwards, the light passes through an acousto-optic modulator (AOM), which works as an optical shutter, to generate intermittent laser with a pulse time of around 100 fs. Such short pulse time leads to a focused laser with high intensity and reduces the chance to burn the material. Then laser beam goes through a neutral density filter and a beam expander. Finally, it passes through an objective lens with high numerical aperture and then focuses on preloaded photosensitive materials. In the meanwhile, the position where the polymerization is activated is controlled by piezoelectric tilt mirrors or galvanometric mirrors and a positioning stage, which are responsible for movements in x, y, and z axes, respectively (**Figure 1**).

Unlike conventional single-photon polymerization, two photons are absorbed simultaneously by photosensitive materials in a typical TPP fabrication process. The total energy from two photons are resonant to the energy difference between ground state and excited state. Owing to characteristics of nonlinear absorption that occurs in this process, TPP has far higher resolution compared with single-photon polymerization.[25] Considering the fact that only the high intensity near focused volume has the capability to convert the solubility of photosensitive materials, the excitation of polymerization is confined within such region of the beam (coined as a voxel), and its size is normally under the limitation of diffraction.

Most photosensitive materials used in TPP are negative-tone photoresists, whose solubility decreases upon exposure to enough dose of photons. This conversion arises from the cross-linking of soluble monomers or oligomers in the photoresist. There are two common intermediates commonly applied in TPP to excite cross-linking: free radicals or cations,[26] and they can be initiated by a photoinitiator or a photoacid generator, respectively. For instance, a photoacid generator is used to initiate cationic polymerization in SU-8,[27] which is a famous type of negative-tone photoresists. However, it only becomes insoluble upon cross-linking of oligomeric epoxide units after receiving enough heat during postbake. To make fabrication simpler, free radical based photoresists have received more attentions in TPP.[28]

At present, acrylates and methacrylates have been widely used in TPP as monomers due to their high activity when radicals present. However, the propagation of such reaction between monomers is unstoppable if no action is applied, resulting in a low resolution. Therefore, oxygen or radical inhibitors are normally used to address this issue. During the process of TPP, another inevitable problem is the shrinkage of the final structure due to the increase of density after cross-linking. Nevertheless, this problem can be solved by offsetting the expected dimensions in advance. Moreover, the increase of refractive index during cross-linking can further cast a shadow over the preciseness of fabrication. Given these concerns, a careful adjustment of different components in the resin including additives such as absorbers and functional materials is inevitable.

3. Treatments of two-photon polymerization for remotely driven micromachines

Although TPP has opened a new door in microfabrication especially for complex 3D structures with high resolution, the polymeric backbones in these structures intrinsically lack mechanical strength and other properties (*e.g.,* conductivity, magnetism, and optical characteristics). Therefore, manipulating the micromachines or their components fabricated via pristine photosensitive materials remotely is still challenging. Nevertheless, various treatments have been developed hitherto to solve this problem, and they can be categorized into two types: material treatments and structural treatments. Obviously, material treatments focus on modifications of photosensitive materials themselves. For instance, ferrofluidic photoresist has been developed by Tian and colleagues through doping synthesized Fe_3O_4 nanoparticles into original photoresist.[29] Therefore, magnetism can be incorporated to final structures without further modifications. Conductive photoresists have also been developed recently by Staudinger and colleagues by dispersing single walled carbon nanotubes into matrix polymer.[30] It is worth noting that these functional composites should be carefully optimized to achieve preferably stable, dispersible, and photopolymerizable properties. Additionally, imparting extra properties may compromise inherent merits of photosensitive materials. For instance, swimmers fabricated by magnetic composites usually have rough surfaces, giving rise to unexpected disturbances under external forces. Consequently, future works that aim to develop new functional materials for TPP should examine various properties comprehensively, and coordinate all components carefully.

On the contrary, structural treatments such as coating usually impart functional properties as subsequent processes after fabrication via TPP. Conventional techniques such as electron-beam physical vapor deposition, chemical vapor deposition, and sputtering are undoubtedly readily available to coat exterior surfaces. For instance, e-beam evaporation was utilized to coat a nickel and titanium (Ni/Ti) bilayer on structures after fabrication via TPP.[31] As one of the ferromagnetic metals, Ni imparted magnetic properties to the structures, and it was the foundation for the remote manipulation of proposed micromachines. Moreover, Bauer and collaborators have incorporated a thin layer of alumina onto exterior surfaces of the final structures by atomic layer deposition (ALD).[32] They have proven that such hybrid structures have a better mechanical strength and recoverability compared with pristine one. Meza and coworkers then furthered removed the polymer backbone after ALD,[33] resulting in a hollow alumina structure, which has been proven to be strong and ductile but light and thin. Additionally, nickel and phosphorus (Ni-P) composite was introduced to the micromachines via electroless plating process.[6] It imparted the micromachines not only the remotely controllable ability but also favorable mechanical performance.

Despite the methods described above have the capabilities of imparting special properties to micromachines, these methods normally do not differentiate the surfaces between polymeric structures and substrates. Under most circumstances, only the functionalization on desired part is demanded and other parts should remain intact. Although particular treatments such as shadowed deposition or use of scarified layers are able to treat only desired regions on structures, they can hardly tackle situations involved complicated 3D shapes. Furthermore, even after washing away soluble photoresists by developer thoroughly, the exterior surface of the polymerized structure still has a low concentration of functional groups (*e.g.*, acrylates, methacrylates or epoxides) that remain unconverted.[34] This trait has enabled selective treatments for structures fabricated by TPP, and unremitting efforts have been made hitherto. To name a few, Chen *et al*. have developed a novel treatment on polymeric structures, and it works for various types of photoresists.[34] Specifically, a lithium aminolysis solution was prepared by mixing *n*-butyllithium and 1, 3-diaminopropane, and it was used to treat remaining groups on external surfaces of fabricated structures. Hereby, amine groups were created in regions where remaining groups were. Gold was subsequently reduced onto surfaces by these groups. At last, silver was incorporated onto gold via electroless deposition. It is critical to note that only the polymeric structure has been deposited with metal while the substrate remained untreated as indicated by different optical reflective properties (**Figure 2**a). In addition to selective treatments between structures and substrates, treatments on specific parts of the structures have also been realized. Farrer and coworkers have creatively fabricated structures using two different types of photoresists (*i.e.*, acrylate and methacrylate based photoresists).[35] They first treated a glass substrate with 3-(Trimethoxysilyl) propyl methacrylate, leaving methacrylate groups on surfaces. Then two photoresists were applied to fabricate different parts one by one, followed by wash with dimethylformamide and ethanol. Finally, the entire structure was immersed into ethylenediamine solution, adding amine groups onto acrylic parts while leaving methacrylic parts unmodified. Hereby selective treatments on specific parts can be realized as amine groups are readily favorable to various chemical treatments. As a demonstration, copper was deposited on an acrylic inductor while the methacrylic support remained unmodified (**Figure 2**b).

As aforementioned, both types of the treatments for TPP have endowed remotely controllable properties to the micromachines as well as enhanced performances such as mechanical strength. Nevertheless, most of these treatments mainly incorporated only one or two properties for micromachines, and they can sometimes compromise other performances. Moreover, the majority of the methods proposed are basically aiming at proof of concept, and only preliminary tests have been done. Thus, a long journey is still ahead to achieve favorable structural treatments for TPP as well as reliable photosensitive materials with special properties.

4. Applications of remotely controllable micromachines fabricated by TPP

At present, mainstream applications with respect to remotely controllable micromachines are still focused on simple structures. Strictly speaking, a microstructure should be complex enough (e.g. contain moving-parts or can do complex tasks) to be qualified for the name “micromachines”. However, the definition of the word has been loosened by many authors to include simpler microstructures. Nevertheless, we see a clear path towards future more complex micromachines based on these studies and therefore use the word “micromachines” for most of the studies discussed here. The remote controlling method of these micromachines can be categorized into several types by means of the physical actuations used. The commonly used types include magnetic, optical, acoustic and other manipulations.

4.1 Magnetic manipulation

Owing to the abilities in non-invasive surgery, bio-objects manipulation and target therapy, micromachines have received ever-increasing attentions in biomedical fields over past years.[36] When it comes to remotely controllable micromachines, magnetic field with low intensity is considered to be biomedically harmless, herein magnetically controllable micromachines has become one of the mainstream studied topics. Given that most biomedical micromachines work in environments where viscosity dominates, and conventional reciprocal movements are difficult to avoid zero net movement. Nevertheless, non-reciprocal movements such as bionic scallop motion and corkscrew motion have addressed this problem.[37] Specifically, a rotational motion is converted to a translational motion using helical structures in viscos environments (**Figure 3**a).[38] Tottori and collaborators have created helical swimming micromachines via TPP recently.[7] In this study, negative-tone photoresists (*i.e.*, SU-8 and IP-L) were used to fabricate helical micromachines, and then a Ni/Ti bilayer was coated on the surface of the micromachines via e-beam evaporation. These two layers are responsible for magnetic actuation and biocompatible insulation, respectively (**Figure 3**b). Finally, the effect of magnetic shape anisotropy was studied under magnetic field, and it was found that smaller helix angle is beneficial as smaller misalignment angle (*i.e.*, angle between external magnetic field and axis of microswimmer) was achieved. A higher frequency of magnetic field also leaded to a smaller wobbled angle (i.e., angle between axes of microswimmer and rotation).

These magnetically driven microswimmers sometimes are also called artificial bacterial flagella (ABF), and various applications have been investigated and presented till now. To name a few, Ding and coworkers have successfully manipulated cells and droplets using ABF.[31] The ABF used were also fabricated by TPP and coated with a Ni/Ti bilayer, then they were detached from the substrate by a sonicator and harvested in a Eppendorf tube. Afterwards, a conventional flow focusing droplet generator was used to encapsulate ABF inside droplets, and the composites can be maneuvered by external magnetic field. Nonetheless, only small droplets that had no contact with walls can be freely maneuvered by magnetic field. Similarly, Suter and coworkers have also created ABF via TPP, but they capitalized on material treatment rather than structural treatment.[39] Photoresist used was prepared by adding Fe_3O_4 nanoparticles into the solvent of SU-8 (*i.e.*, γ-butyrolacton), then the solution was mixed with commercial SU-8. To meet the requirement of viscosity (smaller than $4\times10^{-4}m^2s^{-1}$) for spin coating, the mixture was diluted with γ-butyrolacton. The ABF fabricated have showed a good biocompatibility by WST-1 proliferation assay, and precise manipulation has also been achieved using magnetic field. Peter and collaborators have replaced SU-8 with a novel hydrogel based on polyethylene glycol (PEG),[40] which is more hemocompatible than SU-8 and able to absorb/release drugs. Since pristine PEG lacks the capability in cross-linking, polyethylene glycol diacrylate (PEG-DA) and trimethylolpropane ethoxylate triacrylate (TMPE-TA) that have acrylate groups in the end were used for new photoresist. Superparamagnetic Fe_3O_4 nanoparticles were also included to impart magnetic property. The authors further improved the photoresist and imparted degradability,[14] through which the final structures are hydrolytically degradable, and final products (*i.e.*, poly (acrylic acid) (PAA) and alcoholic moieties) can be easily metabolized and excreted from human body. Another work done by Peter and coworkers has also improved the reliability of ABF. In this study, they developed a novel method to align doped nanoparticles prior to the fabrication.[41] To put it simply, a glass slide coated with a layer of photoresist was placed on a micro hot plate associated with a homemade Helmholtz coil. During the prebake process, a constant magnetic field of 30mT was applied, hence the nanoparticles were aligned along with magnetic field (**Figure 3**c). The ABF fabricated via this method exhibited a wobble-free motion that was difficult to achieve by regular approaches. Different shapes were also investigated later,[42] and it was found that twisted ABF were preferable.

Despite helical microswimmer is still the most widely used micromachine for magnetic control nowadays, other designs have also been investigated and applied. To name a few, a 3D micro-niche has been developed to culture and transport cells to target position using magnetic field gradients.[8] Put simply, cylindrical-shaped and hexahedral-shaped micromachines were fabricated and then coated with a Ni/Ti bilayer. It is found that micromachines with bigger sizes resulted in a decrease of velocity due to a bigger hydrodynamic drag force. But a thicker coating of Ni increases driving force without changing the drag force. Cylindrical-shaped micromachines also showed a faster velocity under same magnetic field gradient compared with hexahedral-shaped ones. Similarly, another porous niche-shaped structure with bigger capacity was proposed by Li and coworkers,[43] and it was found that under a magnetic gradient of 1000 mT/m, the average velocity was able to reach 20 μm/s. Furthermore, a spherical porous shape was also proposed and behaved similar performance.[44]

Other micromachines with magnetic manipulation such as microrotors have also been explored in the past years. For example, Xia and coworkers have presented a microrotor using a ferrofluidic resin composed of methacrylate and Fe_3O_4 nanoparticles (**Figure 4**a).[45] In this study, surface treatment was conducted using 3-(trimethoxysilyl)propyl methacrylate (MPS) to achieve homogeneous dispersion for nanoparticles. Additionally, Tian and coworkers adopted 6-(methacryloyloxy) hexanoic acid to homogenize the mixture nanoparticles and it was stable even under strong magnetic field.[29] A micro-turbine with high good surface flatness was fabricated using the as-prepared mixture. It is found that the rotation speed reached 400 rpm under a rotating magnetic field.[46] Zandrini and collaborators also proposed a magnetic microrotor recently.[47] In this study, Pd catalyst absorption and Fe_3O_4 deposition were utilized to treat acrylic based structures. Specifically, microrotor was immersed inside ethylene diamine solution first, followed by rinse with deionized water. The prepared amine-functionalized structure was then treated by $PdCl_2$ solution, and it was immersed again into NaH_2PO_2 solution. Finally, the microrotor was treated by solution mixed with $Fe(NO_3)_3$ $9H_2O$ and dimethylamine-borane to coat Fe_3O_4 on the surface. As discussed before, it is also possible to selectively treat desired parts of the structure if it was fabricated by different photoresists. Given this concern, the authors have fabricated a magnetite coated rotor with its shaft unmodified as a demonstration. Ni-P alloy was also used to impart remote controllability to micromachines via electroless plating due to the advantages of high hardness, wear resistance, corrosion resistance, and perfect magnetic sensitivity.[6] The hybrid micromachine exhibited great performance in remote manipulation under external magnetic field while maintaining a high mechanical performance (**Figure 4**b).

Wang and collaborators doped the photoresists with surface-modified Fe_3O_4 nanocrystals, and then a micro-spring connected to a sphere was fabricated.[48] Another end of the micro-spring was connected to the substrate with an anchor. Various motions such as bending, swing, and elongation were achieved when an external ferromagnet was used to exert magnetic gradient on the spring. Kim and coworkers have recently proposed a bionic ciliary micromachines, for which a sacrificed mask structure was adopted **(Figure 4**c).[49] After coating the Ni/Ti bilayer on the structure, the mask structure was removed, herein only the cilia part was coated with metal layer. An external magnetic field was then adopted to actuate the cilia using a non-reciprocal motion, which mimicked the motion of a paramecium. It is found that the motion can generate a net propulsive force, pushing the micromachine forward. Similarly, a microtransporter was also developed using TPP and coated with a Ni/Ti bilayer.[50] This syringe-shaped micromachine was developed for delivery of therapeutic agents via corkscrew motion. It is worth noting that various actions such as loading, releasing, and transporting the agents can be achieved in demand.

4.2 Optic and acoustic manipulation

As aforementioned, various micromachines that remotely manipulated by external magnetic field have been developed, including microswimmers, microrotors, microturbines, and other conceptual devices. In spite of the fact that precision manipulation has been realized, pristine photosensitive materials for TPP normally are normally not ferromagnetic. Hence either material treatments or structural treatments are required to impart ferromagnetic property to the micromachines. These treatments not only add complexity in fabrication but may also bring extra issues. For instance, existing ferromagnetic photoresists are usually composed of nanoparticles such as Fe_3O_4, and the stability and homogeneous dispersion usually cannot maintain for a long time. Moreover, resolution and roughness of the micromachines fabricated using these photoresists are not competitive with pristine ones. When it comes to structural treatments, Ni/Ti bilayers are the most widely used coating for magnetic manipulation. Yet nickel is generally deemed as a toxic metal for human beings even though external titanium layer has declared to be biocompatible.

As is evidenced by the disadvantages that described above for magnetic manipulation, numerous studies have been dedicated to investigate other manipulating methods for micromachines, including optic and acoustic manipulation. In contrast with magnetic manipulation, extra treatments are usually not required for these methods. Ever since the single-beam gradient force optical trap was developed by Bell Laboratories in 1986,[51] optic manipulation has been successfully applied in various applications especially in physics, chemistry and biology.[52] This striking technique is also called optical tweezer, and become well-known for its ability in trapping and moving dielectric objects. Generally speaking, the manipulation results from an electric field gradient provided by a highly focused laser beam. When the objects are much smaller than the wavelength of the laser, they are considered as point dipoles as they satisfy Rayleigh scattering. While objects bigger than wavelength of laser are considered as optical lens and the associated manipulation can be explained using ray optics in terms of the conservation of momentum. Herein, objects with various sizes ranging from several nanometers to tens of micrometers are all controllable via optical tweezers in a non-contact, frictionless, and precise way. All these benefits have facilitated the developments of remotely driven micromachines over past few years.

Microscopic objects trapped in a laser beam tend to rotate, and this phenomenon arises from two different mechanisms. Firstly, if the trapped objects have special shapes such as a helical shape, they tend to rotate like a wind turbine rotating against a blast of wind. Secondly, the optical torque originates from the light itself. When the light carries angular momentum, the trapped objects are able to rotate under interactions from the light. To name a few, Galajda and Ormos have demonstrated that complex micromachines can be maneuvered using an optical tweezer.[53] In this study, a light driven microrotor was fabricated by TPP and the optical tweezer was formed by an infrared diode laser focused by an objective lens. The optical tweezer used not only exerted the flux for rotation, but also provided trapping force to hold the microrotor. It is obvious that the rotating momentum exerted depended on the position where the microrotor was focused, and the light scattering also determined the speed of rotation. Herein different shapes would result in different efficiency for microrotors, and the authors found that efficiency of micromachine with shape of sprinkler has surpassed that of micromachines with shapes of helix and propeller.

Furthermore, they also created a propeller that is able to rotate in a reserve direction if the relative position between optical focus and the propeller is changed.[16] Liu and coworkers also applied Archimedes microscrew as a driven tool for the microrotor (**Figure 5**).[54] In this study, when the microscrew was trapped and rotated using optical tweezer, the connected blades were driven together with a high efficiency of 1.9 rpm/mW. As a result, this micromachine could contribute to typical microfluidic applications as a micromixer. Furthermore, micromachines with Archimedes microscrew were adopted by the same group as micropump.[55] Similar to its former study, optical tweezer was applied to trap and rotate the microscrew while the blades connected to the microscrew were used as micropump. The authors found that screw with smaller pitch and thinner blades leaded to a higher rotational speed, and optical tweezers with higher numerical aperture contributed to increase the speed as well. Moreover, microscopic diffractive optical elements have also been incorporated to a micromachine for optical manipulation.[56] By means of incorporating orbital angular momentum to a Gaussian laser beam, any non-absorbing and non-spherical micromachines can be rotated in a conventional optical tweezer system. As a demonstration, a simple symmetric microrotor was fabricated via TPP and manipulated using an optical tweezer (**Figure 6**). It was observed that high torques were generated while the microrotor was trapped.

Nevertheless, micromachines described above were free-standing, hence optical tweezers were adopted not only for generating torques, but also for stabilizing the micromachine to a fixed position. Therefore, only limited motions can be achieved, which hinder its further applications. Maruo and coworkers have demonstrated that micromachines fixed to substrates can perform various motions such as rotating and swinging by an optical tweezer.[9] Put simply, the fabricated micromachine was connected to the substrate, hence no continuous laser was required to keep it at a fixed position. As a demonstrative example, a microgear was fabricated. Specifically, the microgear was kept untethered after fabrication while the shaft was connected to substrate. Torque was then exerted on the microgear using an optical tweezer by partially trapping one tooth of microgear. Herein a controllable laser beam with circular path can drive this constrained micromachine to rotate. Complex motions such as swinging and sliding were also demonstrated using a micromanipulator. Herein, the arm of the micromanipulator was trapped by an optical tweezer that scanned in an arc trajectory. As a result, the micromanipulator was turning around a microobject. Furthermore, the authors developed a micropump based on two microrotors.[57] When two lobed microrotors were driven by a dual optical trapping, a net flow was generated. After releasing the tracer particles into the flow, the authors found that their velocities were proportional to the rotational speed of the microrotors. In addition to the experimental results, total optical torque applied to a microrotor was also measured and compared with results calculated computationally,[58] assuring the contribution of optical manipulation to micromachines. Optically driven microrotor was also used to study hydrodynamic synchronization, by which a self-sustained oscillator was able to maintain stability under perturbations.[59] Specifically, two chiral propellers were fabricated by TPP, and then these counter-spinning propellers were trapped by optical tweezers simultaneously. After adjusting relative torques of two propellers, their patterns were found tending to synchronize with each other. Nevertheless, the coupling force due to hydrodynamic interactions was very weak, hence only small torques should be applied. Ultra-efficient micromachines have also been studied by Ikegami and coworkers recently.[60] Specifically, metallized microrotors were fabricated using TPP, followed by electroless plating. Owing to the repulsive force from reflection at surfaces, the micromachine can be driven by an ultralow power (1mW).

Apart from direct control for micromachines, optical manipulation has also been used to conduct preparing work for micromachines. For instance, free-standing waveguides associated with trapping handles were fabricated using TPP by Palima and coworkers.[61] These waveguides were capable of being manipulated to any direction, thereby providing possibility in sending and collecting light in regions where light otherwise is difficult to reach. Although these waveguides themselves are hardly considered as micromachines, but they can be assembled into a real micromachine in the future, providing optical control. Optical manipulation has also been used for the assembly of micromachines, and its feasibility has been proven by Rodrigo and coworkers through assembly of microscopic puzzle pieces.[62] In this study, shape-complimentary puzzle pieces were fabricated via TPP, and then a programmable multiple-beam optical system was utilized to move and assemble all the pieces into a tessellation (**Figure 7**a). The authors later adopted this method to develop a user-configured microenvironment for studying cellular development processes (**Figure 7**b).[63] Additionally, another optical assembled microrotor was proposed by Köhler and coworkers recently, and it was actuated by magnetic field as a micropump.[64] Other optical manipulating techniques rather than optical tweezer have been investigated as well in the past. To name a few, Kelemen and coworkers incorporated an optical waveguide to maneuver adjacent microrotor.[17] Hereby, complicated setup for an optical tweezer was avoided. Specifically, the optical waveguide and microrotor were fabricated via TPP, and an optical fiber was coupled to the waveguide. As the optical absorbance from the waveguide was negligible, the light from a Verdi laser was able to propagate through and drive the microrotor.

Besides magnetic and optic control, acoustic actuation is another promising method for contactless manipulation, especially for biological applications due to its non-invasiveness. Even though only limited publications have been presented till now, acoustic manipulation of micromachines has still showed a unique advantage over others. For instance, Bertin and coworkers have developed an armed microbubble (AMB) -based microswimmer (**Figure 8**).[10] The shell-shape structure was firstly fabricated on a glass substrate by TPP, then it was placed in a polydimethylsiloxane (PDMS) chamber filled with tracing particles. It is found that the bubble protected by such shell-shape structure can last hours, which circumvent the problems of short lifetime for conventional bubble-based actuation. Afterwards, free-standing microswimmers were designed and fabricated, and they were able to move with a speed of 1mm/s.

5. Conclusion

Two-photon polymerization has opened a new door for the state-of-the-art micromachines in terms of flexible design, complicated structure, high spatial resolution and versatile properties. Over the past decades, great effort has been made to develop innovative micromachines using TPP, and remotely driven ones have also received ever-increasing attentions as well as intensive improvements. In this review, essential principles of TPP has been introduced, and various treatments (material treatments and structural treatments) that are used to impart special properties to micromachines have also been discussed. Moreover, different types of remotely manipulation for micromachines were elucidated.

At present, different methods have been explored and applied to remotely drive micromachines, including magnetic, optic, and acoustic manipulations. Among these methods, magnetic control usually depends on extra treatments to impart ferromagnetic property on micromachines. Thereby, final cost for individual micromachine would increase and compromise the expansion of their applications. Additionally, material treatments such as adding Fe_3O_4 nanoparticles to the photoresists often suffer from problems of stability and homogeneous dispersion. Although the fresh photoresists enable the fabrication, their lifetime can impede the commercialization in the future. When it comes to structural treatments for magnetic manipulation, Ni/Ti bilayer coating is the most widely used technique. However, nickel is a harmful heavy metal to human beings, thereby safer substitutions such as biodegradable or bioresorbable materials are still in demand. Optic manipulation is another commonly used technique in remotely controllable micromachines. In contrast to magnetic control, this method does not require extra treatments as the optical tweezer itself has the ability in trapping and moving microobjects. Nevertheless, as *in vivo* applications usually involve objects with low transmittance, this method is more preferable for microfluidics or prototyping tests in biomedicine fields. Finally, other manipulation techniques such as acoustic manipulation are still in early stage, only a few publications have been presented. This may due to the weak performances that come from materials of TPP. As described above, micromachines fabricated via TPP are solidified voxel by voxel, hence their loose connection may contribute to the weak contributions under acoustic field. On the other hand, even though the acoustically driven microswimmers described above have showed a favorable moving speed, they have to move with the aid of trapped bubbles. Hereby a further study for acoustic manipulation or other methods is inevitable.

In a nut shell, remotely driven micromachines have filled the gap between actionless microstructures and smart microrobots. Different applications such as micromixers, micropumps, microswimmers, and microtransporters have been presented, and it is undoubtedly that they will be expanded in the near future. However, current applications are mainly focused on concept proofing. Most of them are manipulated merely in an ideal environment such as deionized water. But the reality is that they eventually will be applied to a harsher condition such as *in vivo* blood vessels or artificial microfluidic chips. Bulky operation system is also required nowadays for most of the applications. For instance, optical tweezer that used to maneuver micromachines usually requires complicated operations and huge cost. Furthermore, a majority of micromachines lack the possibility in mass production due to extremely high cost and low fabrication speed in a TPP process. Last but not least, automation has not been adopted for remotely controllable micromachines and sophisticated applications (*e.g.*, microrobots) have not been touched yet. Herein, future works with respect to remotely driven micromachines may need to focus on more reliable photoresists associated with biodegradability or bioresorbability, higher TPP fabrication efficiency and stability, and more robust, reliable, affordable and portable operating systems. Besides, one can believe that this bright prospective is around the corner.

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References

[1] M. D. Dickey, K. J. Russell, D. J. Lipomi, V. Narayanamurti, G. M. Whitesides, *small* **2010**, 6, 2050.
[2] R. Kawano, T. Osaki, H. Sasaki, S. Takeuchi, *Small* **2010**, 6, 2100.
[3] E. Ruiz-Hitzky, M. Darder, P. Aranda, K. Ariga, *Advanced materials* **2010**, 22, 323.
[4] K. B. Yesin, K. Vollmers, B. J. Nelson, *The International Journal of Robotics Research* **2006**, 25, 527.
[5] R. Gómez-Martínez, P. Vazquez, M. Duch, A. Muriano, D. Pinacho, N. Sanvicens, F. Sánchez-Baeza, P. Boya, E. J. de la Rosa, J. Esteve, *Small* **2010**, 6, 499.
[6] W.-K. Wang, Z.-B. Sun, M.-L. Zheng, X.-Z. Dong, Z.-S. Zhao, X.-M. Duan, *The Journal of Physical Chemistry C* **2011**, 115, 11275.
[7] S. Tottori, L. Zhang, F. Qiu, K. K. Krawczyk, A. Franco-Obregón, B. J. Nelson, *Advanced materials* **2012**, 24, 811.
[8] S. Kim, F. Qiu, S. Kim, A. Ghanbari, C. Moon, L. Zhang, B. J. Nelson, H. Choi, *Advanced Materials* **2013**, 25, 5863.
[9] S. Maruo, K. Ikuta, H. Korogi, *Journal of Microelectromechanical Systems* **2003**, 12, 533.
[10] N. Bertin, T. A. Spelman, O. Stephan, L. Gredy, M. Bouriau, E. Lauga, P. Marmottant, *Physical Review Applied* **2015**, 4, 064012.
[11] W.-C. Chuang, H.-L. Lee, P.-Z. Chang, Y.-C. Hu, *Sensors* **2010**, 10, 6149.
[12] C. Schizas, V. Melissinaki, A. Gaidukeviciute, C. Reinhardt, C. Ohrt, V. Dedoussis, B. N. Chichkov, C. Fotakis, M. Farsari, D. Karalekas, *The International Journal of Advanced Manufacturing Technology* **2010**, 48, 435.
[13] Y. Tian, Y.-L. Zhang, H. Xia, L. Guo, J.-F. Ku, Y. He, R. Zhang, B.-Z. Xu, Q.-D. Chen, H.-B. Sun, *Physical Chemistry Chemical Physics* **2011**, 13, 4835.

[14] C. Peters, M. Hoop, S. Pané, B. J. Nelson, C. Hierold, *Advanced Materials* **2016**, 28, 533.
[15] D. Palima, J. Glückstad, *Laser & Photonics Reviews* **2013**, 7, 478.
[16] P. Galajda, P. Ormos, *Applied Physics Letters* **2002**, 80, 4653.
[17] L. Kelemen, S. Valkai, P. Ormos, *Applied optics* **2006**, 45, 2777.
[18] K.-S. Lee, R. H. Kim, D.-Y. Yang, S. H. Park, *Progress in Polymer Science* **2008**, 33, 631.
[19] M. Mahjouri-Samani, Y. Zhou, W. Xiong, Y. Gao, M. Mitchell, L. Jiang, Y. Lu, *Nanotechnology* **2010**, 21, 395601.
[20] Z. Zhu, X. Wei, K. Jiang, *Journal of Micromechanics and Microengineering* **2006**, 17, 193.
[21] M. Göppert-Mayer, *Annalen der Physik* **1931**, 401, 273.
[22] W. Kaiser, C. Garrett, *Physical review letters* **1961**, 7, 229.
[23] S. Maruo, O. Nakamura, S. Kawata, *Optics letters* **1997**, 22, 132.
[24] a) A. Marino, C. Filippeschi, V. Mattoli, B. Mazzolai, G. Ciofani, *Nanoscale* **2015**, 7, 2841; b) B.-B. Xu, Y.-L. Zhang, H. Xia, W.-F. Dong, H. Ding, H.-B. Sun, *Lab on a Chip* **2013**, 13, 1677; c) T. Baldacchini, *Three-dimensional Microfabrication Using Two-photon Polymerization: Fundamentals, Technology, and Applications*, William Andrew, **2015**; d) J.-F. Xing, M.-L. Zheng, X.-M. Duan, *Chemical Society Reviews* **2015**, 44, 5031; e) S. Juodkazis, V. Mizeikis, H. Misawa, in *Photoresponsive Polymers I*, Springer **2007**, p. 157; f) H.-B. Sun, S. Kawata, *Advances in Polymer Science* **2004**, 170, 169; g) L. Li, J. T. Fourkas, *Materials Today* **2007**, 10, 30; h) D. Yang, S. J. Jhaveri, C. K. Ober, *MRS bulletin* **2005**, 30, 976; i) M. Rumi, S. Barlow, J. Wang, J. W. Perry, S. R. Marder, in *Photoresponsive Polymers I*, Springer **2008**, p. 1; j) S. H. Park, D. Y. Yang, K. S. Lee, *Laser & Photonics Reviews* **2009**, 3, 1; k) M. T. Raimondi, S. M. Eaton, M. M. Nava, M. Laganà, G. Cerullo, R. Osellame, *J Appl Biomater Biomech* **2012**, 10, 55; l) Y.-L. Zhang, Q.-D. Chen, H. Xia, H.-B. Sun, *Nano Today* **2010**, 5, 435; m) D. D. Han, Y. L. Zhang, J. N. Ma, Y. Q. Liu, B. Han, H. B. Sun, *Advanced Materials* **2016**, 28, 8328.
[25] K. Sugioka, J. Xu, D. Wu, Y. Hanada, Z. Wang, Y. Cheng, K. Midorikawa, *Lab on a Chip* **2014**, 14, 3447.
[26] A. Selimis, M. Farsari, *Multiphoton Lithography: Techniques, Materials, and Applications* **2016**, 1812.
[27] S. Juodkazis, V. Mizeikis, K. K. Seet, M. Miwa, H. Misawa, *Nanotechnology* **2005**, 16, 846.
[28] N. C. Strandwitz, A. Khan, S. W. Boettcher, A. A. Mikhailovsky, C. J. Hawker, T.-Q. Nguyen, G. D. Stucky, *Journal of the American Chemical Society* **2008**, 130, 8280.
[29] Y. Tian, D. Lu, H. Jiang, X. Lin, *Journal of Magnetism and Magnetic Materials* **2012**, 324, 3291.
[30] U. Staudinger, G. Zyla, B. Krause, A. Janke, D. Fischer, C. Esen, B. Voit, A. Ostendorf, *Microelectronic Engineering* **2017**, 179, 48.
[31] Y. Ding, F. Qiu, X. Casadevall i Solvas, F. W. Y. Chiu, B. J. Nelson, A. deMello, *Micromachines* **2016**, 7, 25.
[32] J. Bauer, S. Hengsbach, I. Tesari, R. Schwaiger, O. Kraft, *Proceedings of the National Academy of Sciences* **2014**, 111, 2453.
[33] L. R. Meza, S. Das, J. R. Greer, *Science* **2014**, 345, 1322.
[34] Y. S. Chen, A. Tal, D. B. Torrance, S. M. Kuebler, *Advanced Functional Materials* **2006**, 16, 1739.
[35] R. A. Farrer, C. N. LaFratta, L. Li, J. Praino, M. J. Naughton, B. E. Saleh, M. C. Teich, J. T. Fourkas, *Journal of the American Chemical Society* **2006**, 128, 1796.
[36] B. J. Nelson, I. K. Kaliakatsos, J. J. Abbott, *Annual review of biomedical engineering* **2010**, 12, 55.
[37] a) E. M. Purcell, *American journal of physics* **1977**, 45, 3; b) W. R. DiLuzio, L. Turner, M. Mayer, P. Garstecki, D. B. Weibel, H. C. Berg, G. M. Whitesides, *Nature* **2005**, 435, 1271.
[38] K. E. Peyer, L. Zhang, B. J. Nelson, *Nanoscale* **2013**, 5, 1259.
[39] M. Suter, L. Zhang, E. C. Siringil, C. Peters, T. Luehmann, O. Ergeneman, K. E. Peyer, B. J. Nelson, C. Hierold, *Biomedical microdevices* **2013**, 15, 997.

[40] C. Peters, V. Costanza, S. Pane, B. J. Nelson, C. Hierold, presented at Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS), 2015 Transducers-2015 18th International Conference on **2015**.
[41] C. Peters, O. Ergeneman, B. J. Nelson, C. Hierold, presented at Micro Electro Mechanical Systems (MEMS), 2013 IEEE 26th International Conference on **2013**.
[42] C. Peters, O. Ergeneman, P. D. W. García, M. Müller, S. Pané, B. J. Nelson, C. Hierold, *Advanced Functional Materials* **2014**, 24, 5269.
[43] J. Li, F. Niu, Y. Chow, S. Chen, D. Sun, presented at Nanotechnology (IEEE-NANO), 2015 IEEE 15th International Conference on **2015**.
[44] J. Li, W. Ma, F. Niu, Y. T. Chow, S. Chen, B. Ouyang, H. Ji, J. Yang, D. Sun, presented at Advanced Intelligent Mechatronics (AIM), 2016 IEEE International Conference on **2016**.
[45] H. Xia, J. Wang, Y. Tian, Q. D. Chen, X. B. Du, Y. L. Zhang, Y. He, H. B. Sun, *Advanced Materials* **2010**, 22, 3204.
[46] Y. Tian, X. Shao, *Journal of Nanomaterials* **2016**, 2016.
[47] T. Zandrini, S. Taniguchi, S. Maruo, *Micromachines* **2017**, 8, 35.
[48] J. Wang, H. Xia, B.-B. Xu, L.-G. Niu, D. Wu, Q.-D. Chen, H.-B. Sun, *Optics letters* **2009**, 34, 581.
[49] S. Kim, S. Lee, J. Lee, B. J. Nelson, L. Zhang, H. Choi, *Scientific reports* **2016**, 6, 30713.
[50] T. Y. Huang, M. S. Sakar, A. Mao, A. J. Petruska, F. Qiu, X. B. Chen, S. Kennedy, D. Mooney, B. J. Nelson, *Advanced Materials* **2015**, 27, 6644.
[51] A. Ashkin, J. M. Dziedzic, J. Bjorkholm, S. Chu, *Optics letters* **1986**, 11, 288.
[52] D. G. Grier, *Nature* **2003**, 424, 810.
[53] P. Galajda, P. Ormos, presented at Lasers and Electro-Optics, 2002. CLEO'02. Technical Digest. Summaries of Papers Presented at the **2002**.
[54] Y.-J. Liu, Y.-H. Lee, Y.-S. Lin, C. Tsou, P. L. Baldeck, C.-L. Lin, presented at Actuators **2013**.
[55] C.-L. Lin, Y.-S. Lin, P. L. Baldeck, *Micromachines* **2015**, 6, 674.
[56] a) G. Knöner, S. Parkin, T. A. Nieminen, V. L. Loke, N. R. Heckenberg, H. Rubinsztein-Dunlop, *Optics express* **2007**, 15, 5521; b) S. Ganchevskaya, R. Skidanov, presented at CEUR Workshop Proceedings **2016**.
[57] S. Maruo, H. Inoue, *Applied Physics Letters* **2006**, 89, 144101.
[58] T. Asavei, V. L. Loke, M. Barbieri, T. A. Nieminen, N. R. Heckenberg, H. Rubinsztein-Dunlop, *New Journal of Physics* **2009**, 11, 093021.
[59] R. Di Leonardo, A. Búzás, L. Kelemen, G. Vizsnyiczai, L. Oroszi, P. Ormos, *Physical review letters* **2012**, 109, 034104.
[60] a) T. Ikegami, R. Ozawa, M. Stocker, J. Fourkas, S. Maruo, presented at 16th International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS 2012 **2012**; b) T. Ikegami, R. Ozawa, M. P. Stocker, K. Monaco, J. T. Fourkas, S. Maruo, *Journal of Laser Micro Nanoengineering* **2013**, 8, 6.
[61] D. Palima, A. R. Bañas, G. Vizsnyiczai, L. Kelemen, P. Ormos, J. Glückstad, *Optics express* **2012**, 20, 2004.
[62] P. J. Rodrigo, L. Kelemen, C. A. Alonzo, I. R. Perch-Nielsen, J. S. Dam, P. Ormos, J. Glückstad, *Optics express* **2007**, 15, 9009.
[63] P. J. Rodrigo, L. Kelemen, D. Palima, C. A. Alonzo, P. Ormos, J. Glückstad, *Optics Express* **2009**, 17, 6578.
[64] J. Köhler, R. Ghadiri, S. Ksouri, Q. Guo, E. Gurevich, A. Ostendorf, *Journal of Physics D: Applied Physics* **2014**, 47, 505501.

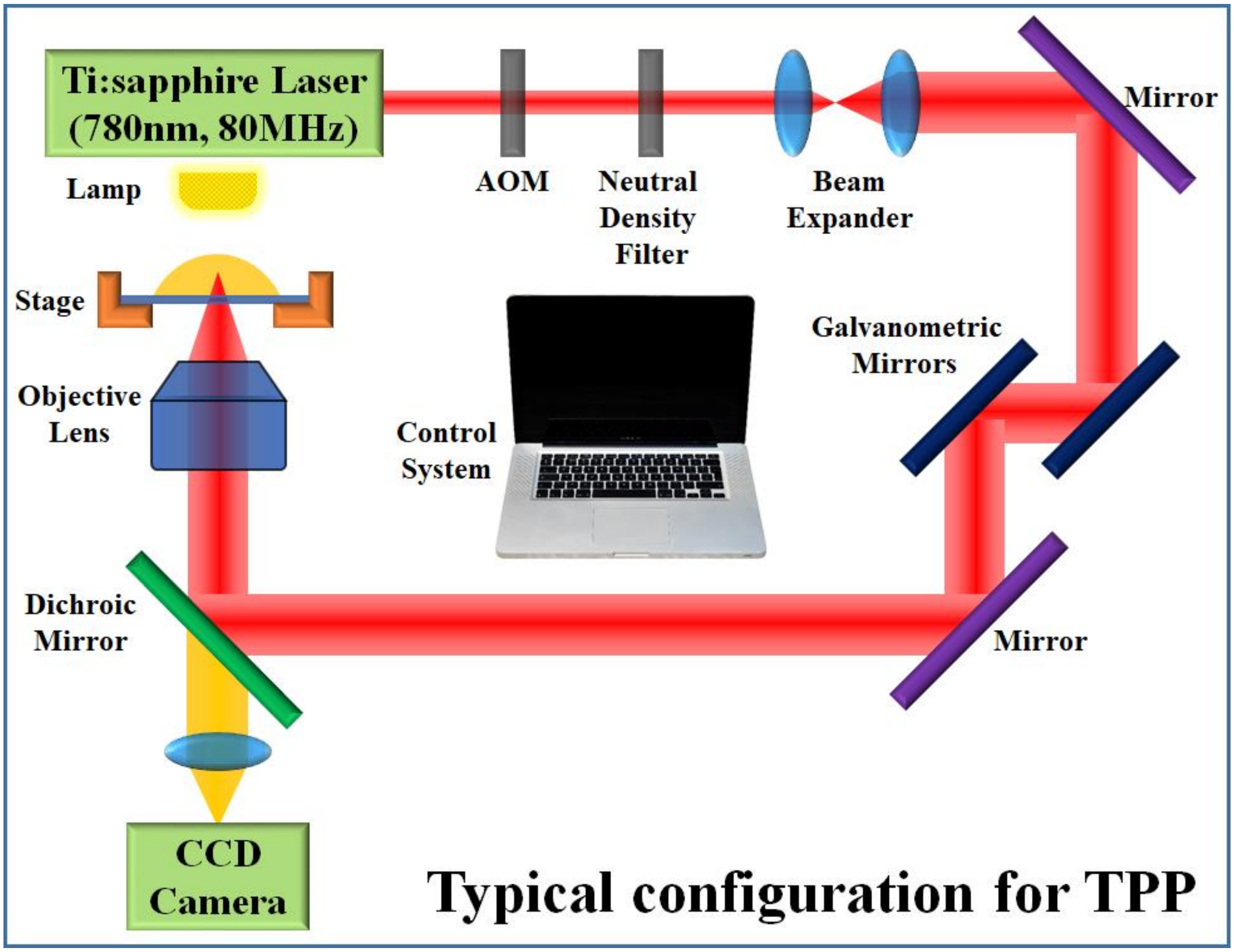

Figure 1. Schematic illustration of the configuration for a typical TPP process. First, a Ti:sapphire laser is used to generate consistent light with wavelength around 780nm. Afterwards, the light passes through an optical shutter, followed by a neutral density filter and a beam expander. Finally, it focuses on photosensitive materials through an objective lens. In the meanwhile, two galvanometric mirrors and a positioning stage are used to control the movements in x, y, and z axes, respectively.

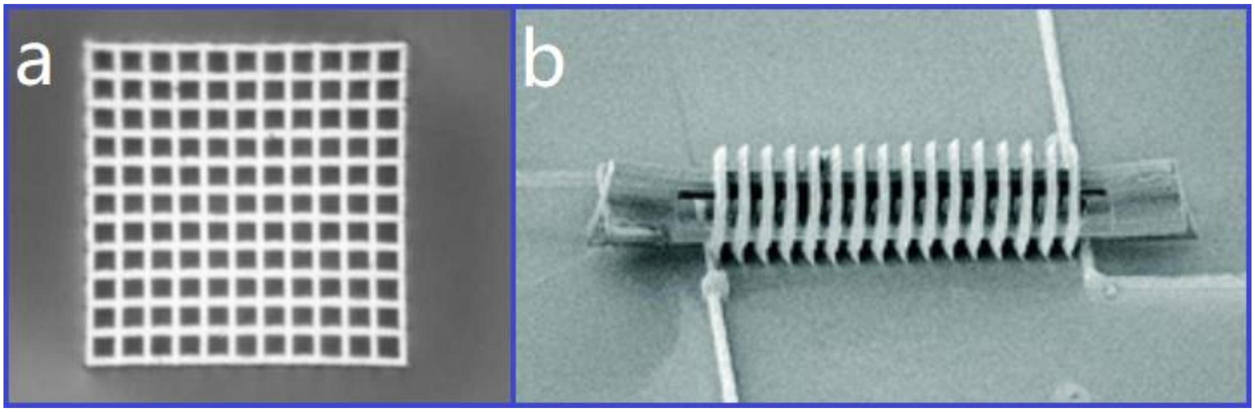

Figure 2. a) Optically reflective proof for selectively functionalization on polymeric structure rather than substrate. Adapted with permission.[34] Copyright 2016, John Wiley & Sons. b) Copper coated acrylic inductor and unmodified methacrylic support. Adapted with permission.[35] Copyright 2006, American Chemical Society.

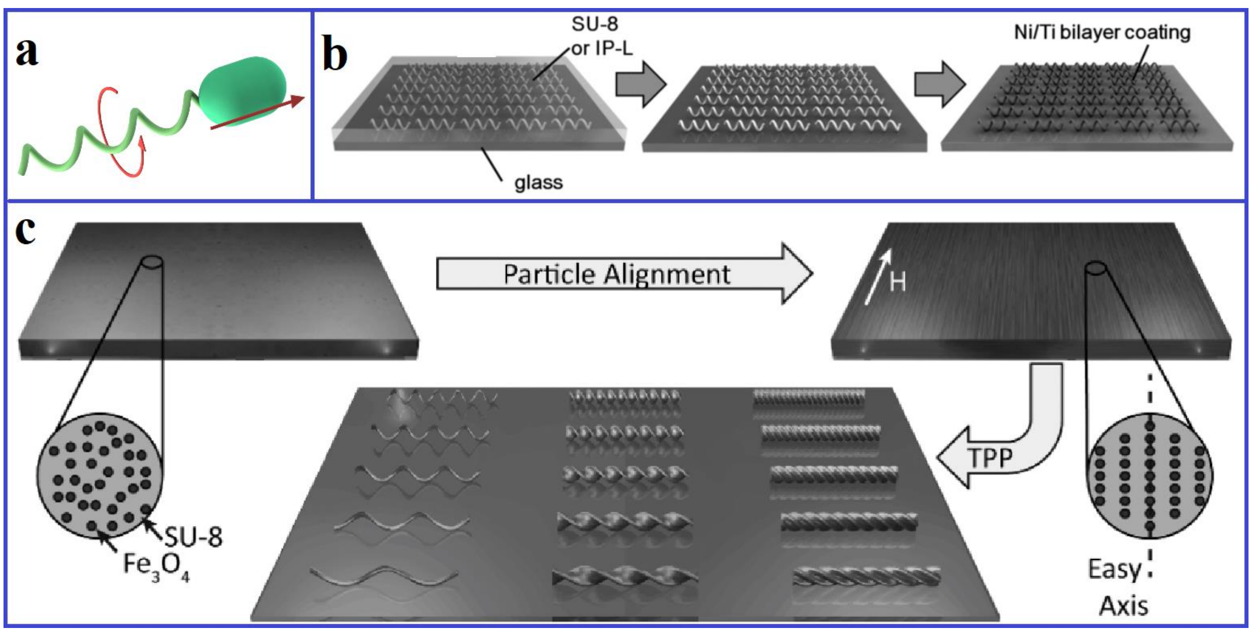

Figure 3. a) Schematic illustration of the corkscrew motion. A rotational motion is converted to a translational motion. b) Fabrication process of helical swimming micromachines by TPP and e-beam evaporation. Adapted with permission.[7] Copyright 2012, John Wiley & Sons. c) Particle

alignment before TPP process for easy-axis. Adapted with permission.[42] Copyright 2014, John Wiley & Sons.

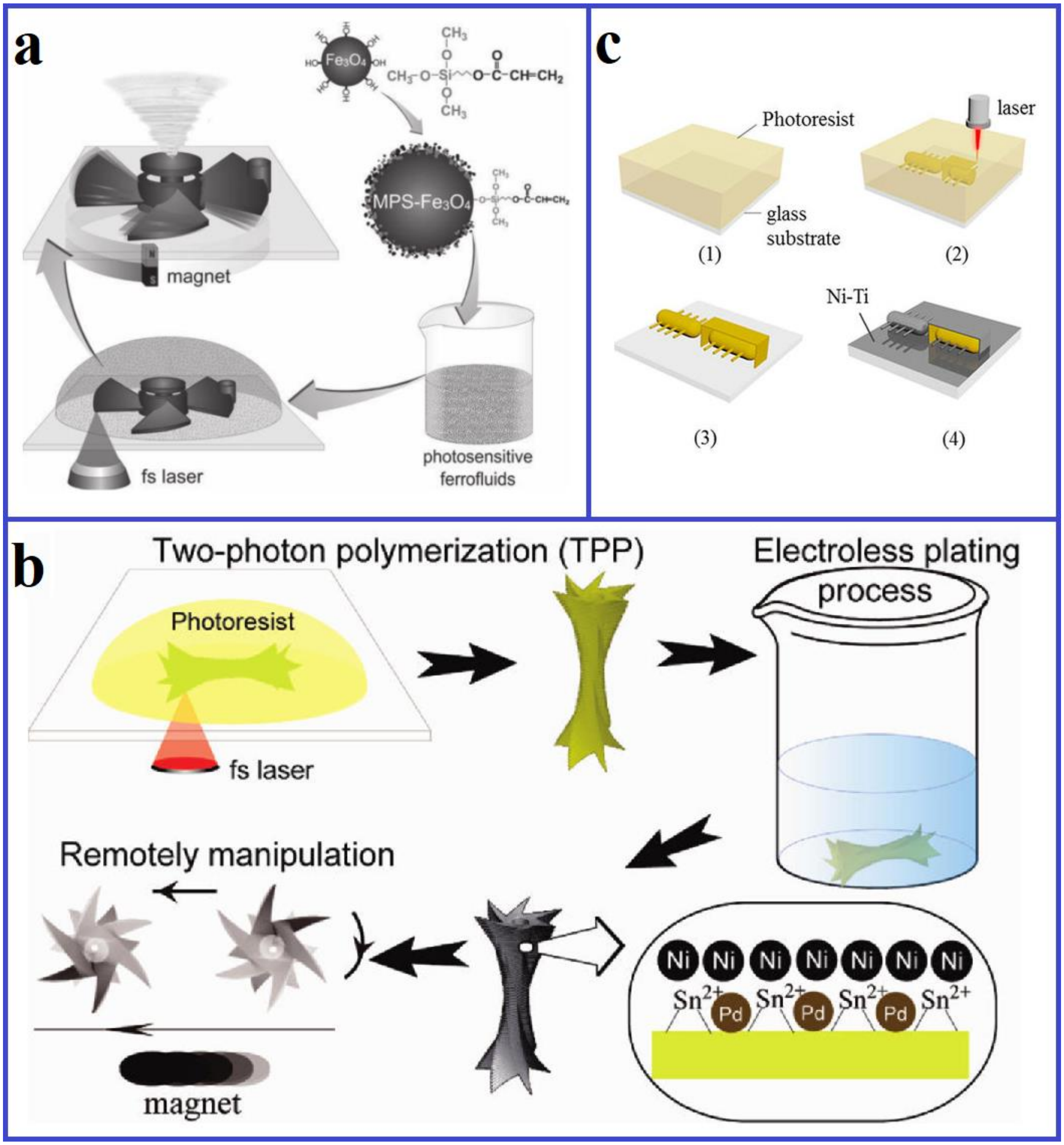

Figure 4. a) Schematic illustration for the fabrication process of a microrotor using novel ferrofluidic resin based on Fe_3O_4 nanoparticles. Adapted with permission.[45] Copyright 2010, John

Wiley & Sons. b) Selective coating of Ni-P alloy to a microrotor while leaving its shaft unmodified via electroless plating. Adapted with permission.[6] Copyright 2011, American Chemical Society. c) Scheme of the fabrication process for a bionic paramecium with the aid of a sacrificial layer. Adapted with permission.[49] Copyright 2016, Nature.

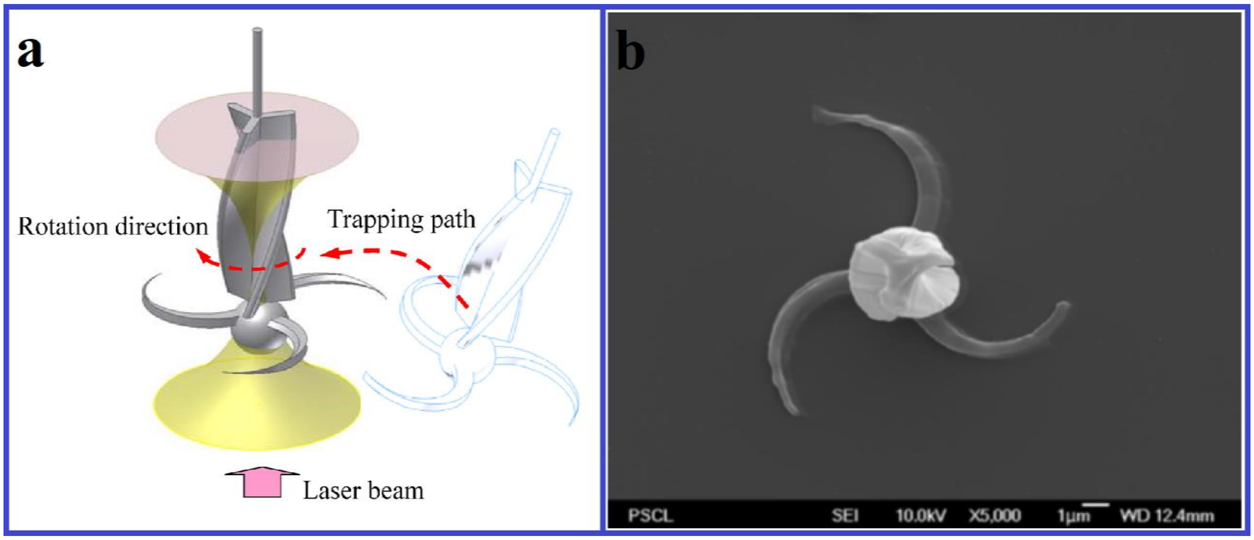

Figure 5. a) Schematic illustration for an optically driven micromachine based on Archimedes screw, which was automatically driven by the optical tweezer when the main body was trapped. b) SEM image for the as-prepared micromachine. Adapted with permission.[54] Copyright 2013, MDPI.

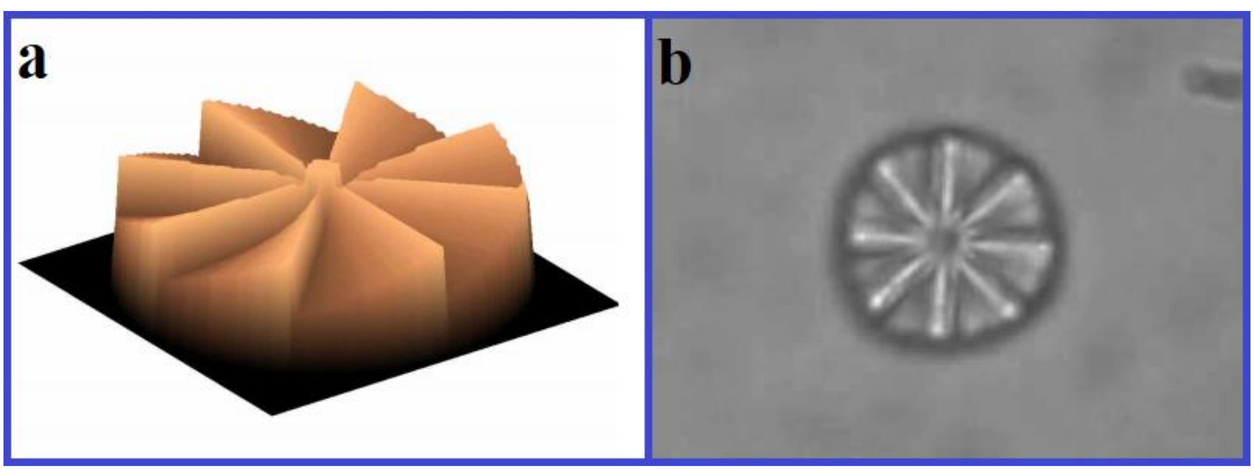

Figure 6. Optically driven micromachine based on a microscopic diffractive optical element. a) A 3D model for the micromachine used to produce orbital angular momentum via a Gaussian laser beam. b) Image of the micromachine fabricated by TPP. Adapted with permission.[56a] Copyright 2013, MDPI.

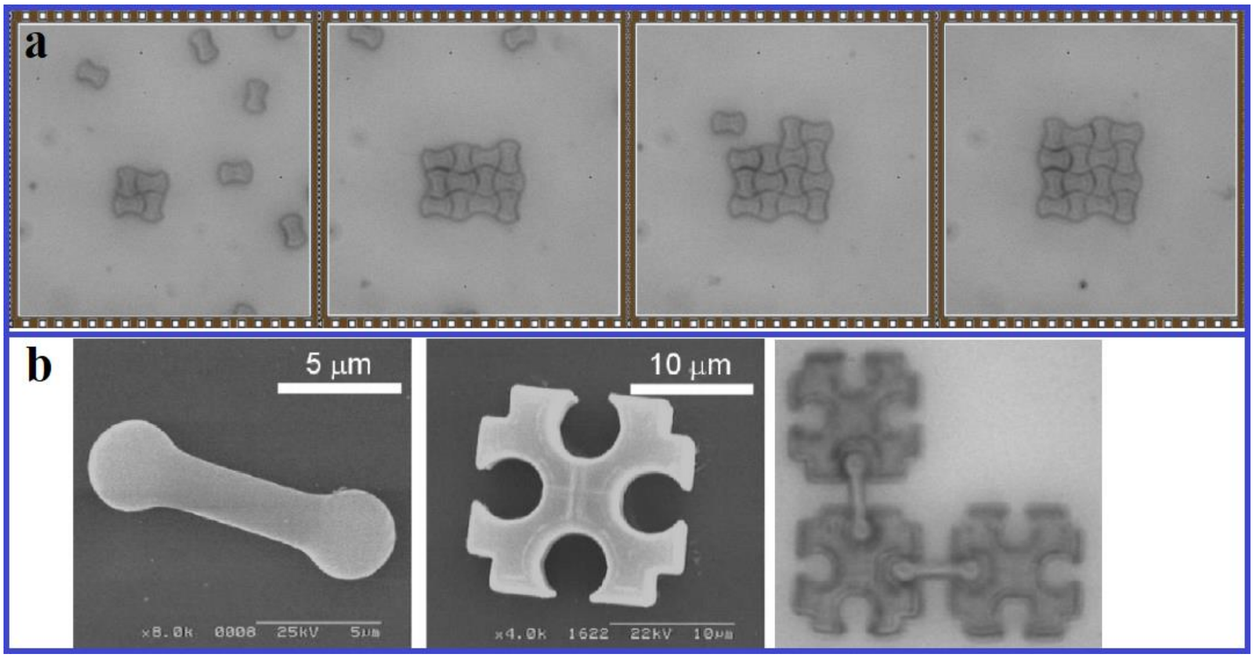

Figure 7. a) Photos of assembling puzzle pieces into a 4x4 tessellation using optical manipulation. Adapted with permission.[62] Copyright 2007, OSA Publishing. b) User-reconfigurable biological platform based on optical assembly. Adapted with permission.[63] Copyright 2009, OSA Publishing.

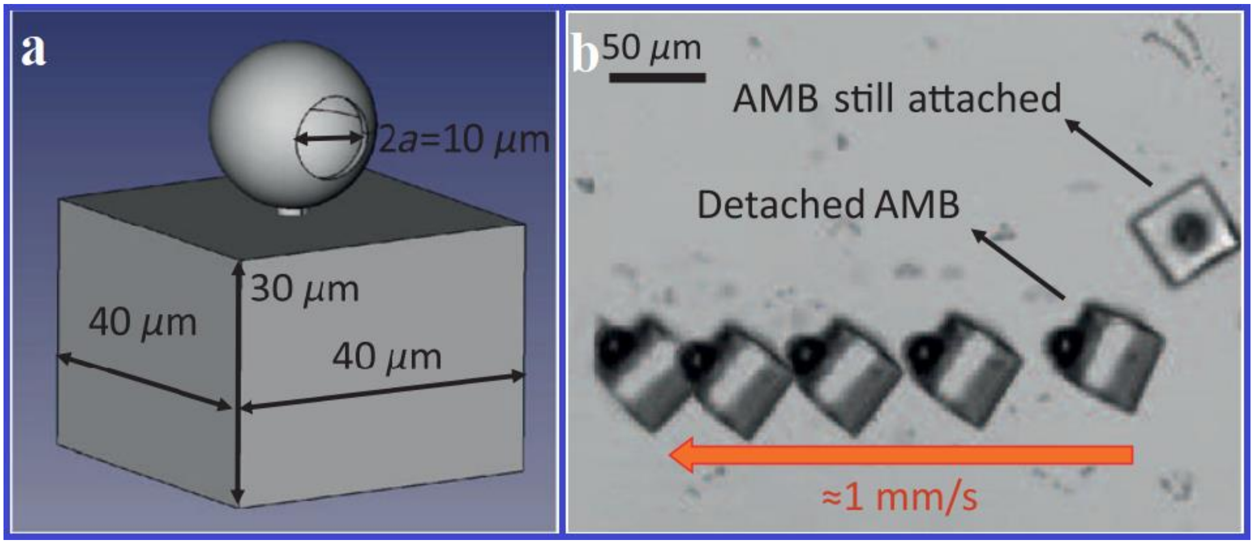

Figure 8. Acoustically driven microswimmer based on armed microbubble (AMB) a) Scheme of a 3D model for a AMB-based microswimmer. b) Photo of manipulating microswimmer with an acoustic pressure of 5.6 kPa. Adapted with permission.[10] Copyright 2015, American Physical Society.