Fabrication and Optical Manipulation of Microrobots for Biomedical Applications

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Abstract

Optical manipulation is a technology that enables accurate manipulation of microrobots in fluidic environment. Optical microrobots, which can be used as micro-tools to perform indirect micro-objects manipulation via optical tweezer (OT) have been employed for various biomedical applications. Supported by the latest advances in 3D micro-fabrication, microrobots with sophisticated structures can be created with good reproducibility and yield, further expanding the use of OT for manipulating microrobots. In this review, emerging concepts and recent progress of allied techniques (e.g. micro-fabrication and materials), as well as the general trend of optical microrobots, are introduced. Technically, the paper also covers visual perception and manipulation of microrobots with OT. We further highlight open research challenges and future research directions.

1. Introduction

With the advent of different micro/nano fabrication techniques supported by advances in nanotechnology, biotechnology, and materials science, micro-robotics has attracted extensive interests in recent years [1]. Microrobots can be actuated by magnetic, acoustic, chemical, electrical, or optical fields. Among all the actuation methods, magnetic and optical manipulation approaches are the most popular ones for biomedical applications. Magnetic actuation has the advantage of high penetration depths when applied to operating microrobots inside nontransparent tissues. The advantage of optical manipulation is its high spatial selectivity. It is known as one of the most precise manipulation techniques [2]. The light field can be decentralized to control either multiple microrobots independently or simultaneously [3, 4]. This review focuses on optical manipulation, and discussions about other micro-manipulation techniques can be found in Zhang et al [5].

Optical microrobots can be fabricated by using shape-changing materials, such as light sensitive polymers. The deformation of the materials can lead to deformation of microrobots' body components, and therefore control the movements of microrobots. However, this mechanism is difficult to enable six Degrees-of-Freedom (DoFs) dexterous manipulation of micro-robots as material properties can be nonlinear, which is difficult to ensure the precision for closed-loop control.

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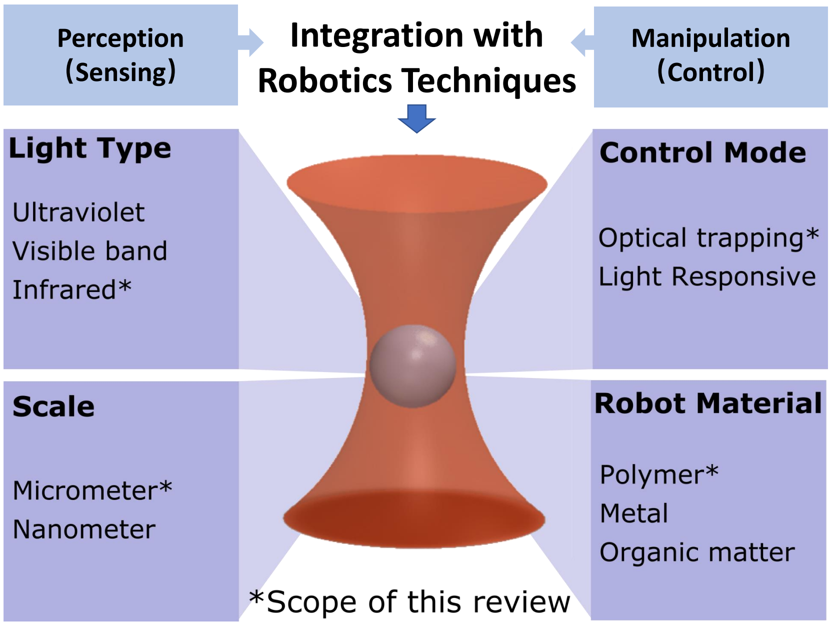


Figure 1: Illustration of the scope of the review: OT based indirect manipulation of micro-objects using optical microrobots, while the main focus of this review lies in the perception and manipulation of optical robots for biomedical applications.

Another popular optical manipulation method is to leverage the momentum carried by laser beams to enable optical trapping of micro-objects, which is the basis of optical tweezers (OT) [6]. When the scale is below 100 of microns, the force generated by light-induced mechanical effects becomes significant. The basic principles of OT are introduced in [7]. The precision of operating microrobots using OT-based methods is much higher than that of microrobots actuated by light-induced shape-changing materials. The dimension of OT actuated microrobots can be several microns to tens of microns, while the dimension of light-induced shape-changing microrobots is usually tens or hundreds of microns. For indirect manipulation of biological objects with sizes of several microns (such as cells), OT-based microrobots are more suitable.

Optical microrobots bring together advances in photonics and robotics to enable precise and dexterous manipulation at micro-scales [8] by using light for trapping and manipulation. In this review, the term optical microrobots refer to 3D fabricated synthetic micro-components or micro-devices that can be actuated by light or laser beams at microscales. These micro-components or micro-devices are also termed as light/optically driven, light/optically-controlled, light/optically-powered, or light/optically-actuated microrobots in other literature. It is worth noting that the name ‘microrobots’ is sometimes referred to by other terms, such as ‘microtools’, ‘micromotors’, ‘microswimmers’, or ‘micromachines’ in other literature. This review mainly focuses on micromanipulation using OTs from a robotics perspective. The use of robotics and AI techniques can accelerate the deployment of optical manipulation in a wide range of applications, especially in biotechnology and lab-on-a-chip applications.

The integration of robotic techniques with optical manipulation is challenging. Optical micro-robotics is not a simple scaled-down version of macroscale robots, since physical effects in macro and micro worlds are significantly different [9]. Moreover, there are many technical challenges arising from miniaturization. For example, it is difficult to fabricate and transform existing robotics techniques from macro-scale to micro-scale. Similar to their macroscale counterparts, precise perception and dexterous manipulation are two major aspects that need to be considered for

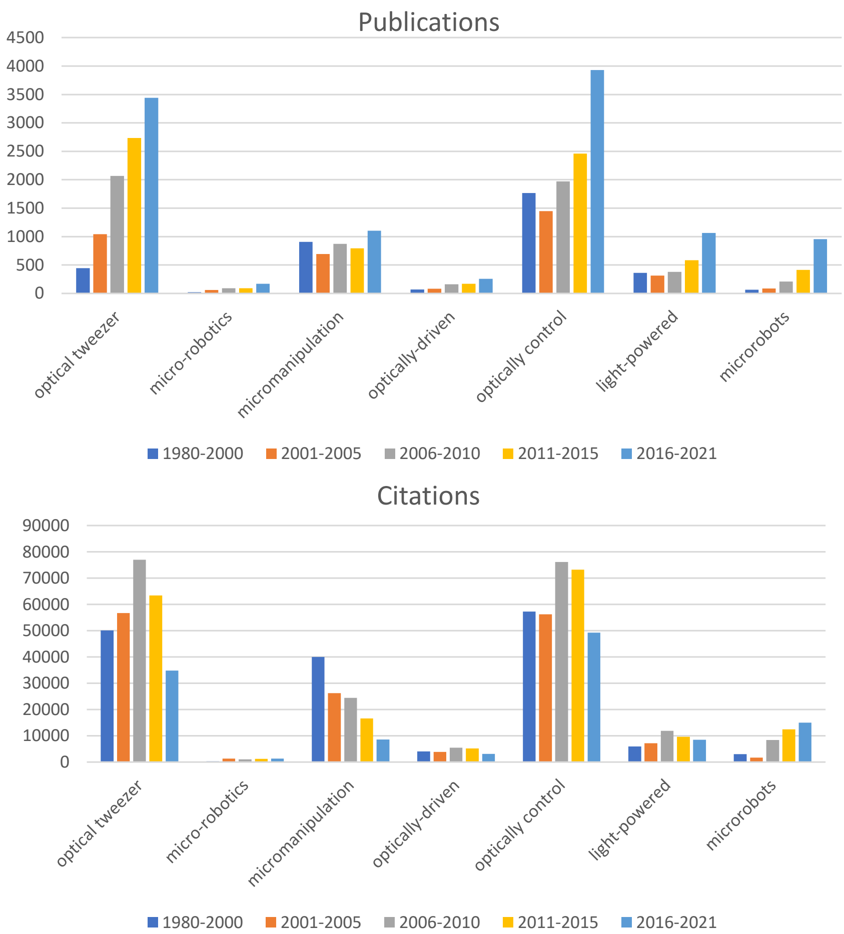


Figure 2: Number of publications and citations containing the terms “optical tweezer,” “micro-robotics,” “micromanipulation,” “optically-driven,” “optically-control,” “light-powered,” and “microrobot” from January 1980 to the December 15, 2021, according to Google Scholar.

micro-robotics, which will be detailed in this review. Robotic perception includes sensory data processing (imaging, detection and tracking), data analysis and interpretation (depth and pose estimation of microrobots), which are the prerequisites for planning and manipulation of microrobots for task execution. The tracking and detection of microrobots can enable the OT to trap the microrobots for indirect manipulation of target biological objects with high efficiency. Moreover, with accurate pose and depth estimation of micro-robots based on data obtained from the sensing system, the operators can have a better understanding of the operation scene and can know how to control the target object to the desired position and pose for operation. This also paves a way for the construction of a feedback control system for robot-assisted optical manipulation. Moreover, dexterous manipulation is essential for the microrobots to conduct complex operations in biomedical applications, for example to assist cell manipulation and micro-surgery.

Fig. 1 summarizes the scope of the review. The literature search for this review was conducted by using Web of Science, Google Scholar, and IEEE Xplore. The keywords include the combination of “optical tweezer,” “micro-robotics,” “micromanipulation,” “optically driven,” “optically-control,” “light-powered,” and “microrobot”. Fig. 2 shows the number of relevant publications with the terms mentioned above from 1980 to the end of 2021.

In the rest of this paper, we first introduce the basic concepts of optical manipulation, including the implementation of multiple optical traps in Section 2. The current limitations of direct manipulation of micro-objects using OT and the challenges associated with indirect manipulation are illustrated. The state-of-the-art research related to the perception and manipulation of optical microrobots via OT is presented. Subsequently, relevant techniques are introduced in Section 3 about the current limitations of 3D imaging, visualization for the optical micro-robotic system, and multi-DoF pose estimation. Dexterous, indirect micro-object manipulation using optical microrobots with complex shapes is addressed in Section 4. Following from that, we discuss optical microrobots for applications in cell manipulation, characterization, micro-assembly in Section 5. A future roadmap is provided in Section 6 for the development of optical microrobots for biomedical applications. Finally, conclusions are drawn while the future outlook is provided in Section 7, highlighting the need of multidisciplinary collaboration among optics, materials, robotics and automation science.

2. Fundamentals of Optical Trapping and Current Limitations

In this section, the basic principles of optical trapping and a concise description of OT are provided. Two typical types of setups for multiple optical trap generation are introduced, while the current limitations of perception and manipulation of optical micro-robotic systems are illustrated.

2.1. Optical Tweezer

Ashkin et al first introduced stable optical trapping via a single tightly focused laser beam in 1986 [10]. The laser beam can enable micro-object manipulation in sealed and sterile biological chambers. This optical trapping technique is named OT later, which is developed based on photon linear momentum transfer and can be used for trapping and manipulation of biological samples or micro-objects without physical contact [11].

In most OT systems, a high numerical aperture (NA) objective is normally used to focus the laser beam to spaces within a narrow region, where a gradient and a scattering force are formed to trap micro-object at the equilibrium point, The optical force ranges from 10^{-14} to 10^{-10} Newtons for trapping micro-objects [6], while the scales of the trapped micro-objects are normally from 10s of nanometres to 100s of micrometers.

2.2. OT with Multiple Optical Traps

To control the overall position of the microrobot and its relative motions, multiple optical traps are necessary [12]. Using multiple laser beams is expensive and occupies more space due to the increased complexity of the optical experimental setups. Generating multi-traps from a single focused laser beam is a better strategy. Here, two common approaches used in practice are introduced,through which a laser beam with multi-spots can be used to manipulate multiple microrobots simultaneously.

Fig. 3 (a) illustrates the experimental setup of a conventional OT system with a time-sharing setup and the corresponding schematic diagram of optics in the system. Time-sharing setups for OT typically use fast-switching acousto-optic deflectors (AOD) or electro-optic deflectors (EOD) to deflect the beam and control the rapid movement of the laser beam. The frequency is high, so multiple optical traps can be generated using this approach [15].

For most OT systems with the time-sharing setup, the spatial position control with an OT is straightforward and several micro-objects can be trapped and transported to the target areas. Trapping multiple parts of a structure via different focal points has been used to control the planar rotation of a microrobot [16]. However, out-of-plane orientation control is difficult to implement, since the focal points of the optical laser traps are constrained in the same

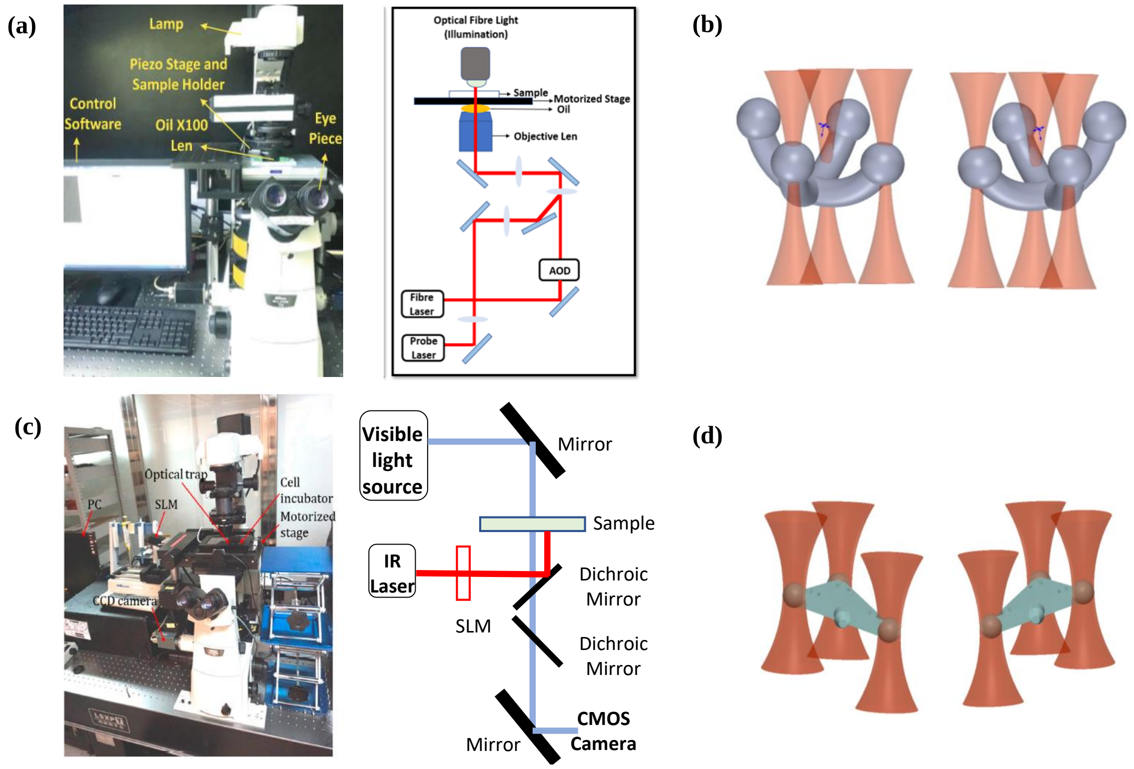


Figure 3: OT system and a schematic diagram of optics, and the concept illustration of optical microrobot manipulation via different OT setups. (a) The conventional OT system which can generate multiple laser spots based on time-sharing; the schematic diagram of optics for the corresponding OT system. (b) The concept illustration of dexterous manipulation of optical microrobots using conventional OT systems. (c) The conventional holographic optical tweezer (HOT) system which can generate multiple laser spots; the schematic diagram of optics for the corresponding HOT system. Image obtained from [13]. (d) The concept illustration of dexterous manipulation of optical microrobots using HOT system. Panel (a) reproduced from [14] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Wiley. Panel (c) reproduced from [13] with permission. Copyright 2019, IEEE.

focal plane, while the special design of the microrobot is required to achieve out-of-plane manipulation (see Fig. 3 (b)).

Fig. 3 (c) shows a schematic diagram of a typical Holographic Optical Tweezer (HOT) system as well as the corresponding optics. HOT is a type of OT that can generate multiple traps, where Spatial Light Modulators (SLM) are used to split an incoming laser beam to form multiple laser traps [17]. The laser beams are directed through the microscope aperture to form multiple optical traps. The number of traps and the range of movement depends on the SLM, and can be adapted to different operation requirements. In such a setting, as each optical trap can be controlled independently, they are not constrained to be co-planar. Out-of-plane manipulation of micro-objects can therefore be achieved with ease, as illustrated in Fig. 3 (d). However, the control of optical microrobot via HOT is more time-intensive, compared to time-sharing OT, which limits its real-time application. Therefore, the trade-off between performance and speed should be considered depending on different application requirements [18].

2.3. Current Limitations and Challenges

2.3.1. Limitations

Though light can exploit momentum to trap and move micro-objects without contacts, it also introduces secondary effects, such as heat and phototoxicity. Excessive heat generated by intense laser light may cause damages to micro-objects or specimens being studied during manipulation [19]. To reduce optical damage caused by the laser when using OT, altering trapping wavelength or utilizing radical scavenging species have been considered [20]. However, these methods require changing the experimental setups and may add external constraints to the experiments. To adapt to different experimental conditions, one can reduce the power of the laser. However, the generated forces for actuation will be reduced in the meantime, which limits relevant research that requires high force, such as DNA overstretches.

Moreover, direct trapping of the target object may not always be possible, since the target objects' features may not meet the conditions for stable optical trapping. In addition to light-induced heat, current OT systems for indirect manipulation also have other inherent limitations. These include visual perception and manipulation.

2.3.2. Challenges of Perception

Optical manipulation experiments normally rely on a single camera view for the tracking of the microrobots or for characterization. Due to the challenges of micro-fabrication, time-of-flight cameras, stereo cameras, structured illumination etc are widely used for large-scale robotic systems but cannot be used for microscale robotic systems. Moreover, due to the parallel projection for imaging using optical microscope [21], the motions of the microrobots parallel to the optical axis are difficult to be observed [22]. In addition, the transparency of optical microrobots and varying illumination levels set by the users may bring additional challenges to depth estimation. Compared to opaque objects, a transparent object may complicate. The sharpness variance may bring extra challenges when using the existing vision techniques.

Therefore, reliable vision processing is essential for the development of a robust robot-assisted OT system. The vision system for microrobots is essential for fully automated, simultaneous, independent manipulation of multiple microrobots with complex shapes.

2.3.3. Challenges of Manipulation

For conventional OT, manipulation of microrobots in 3D is difficult. The target object for operation via conventional OT only has planar rotation and translational movements. For biological object manipulation, the orientation control is important [13]. For example, the control of the rotation angle of a cell is necessary for detailed structural and functional characterization [23]. The cells are also required to be rotated to maintain an appropriate orientation for cell surgery.

For example, during the injection of nucleic acid or other exogenous materials into a cell, the cell orientation must be controlled to avoid collateral damage to other structures. Oocytes need to be oriented properly to enable cell membrane perforation for in vitro fertilization (IVF) [24]. To visualize the polar body, organelle, or other structures of a cell with a microscope for procedures such as preimplantation genetic diagnosis (PGD) [25], intracytoplasmic sperm injection (ICSI) [26], organelle extraction analysis and diagnosis [27], the cells also need to be oriented properly.

The precision of orientation control for the target object using traditional methods is limited, since manual manipulation errors may occur. For example, two micro-pipettes are required to control the orientation of a mature oocyte for intracytoplasmic sperm injection [28]. The use of microrobots for indirect manipulation can help increase the precision for manipulation and enhance the efficiency for operation [29]. To this end, developing microrobots that have dexterous 6D manipulation of micro-objects in 3D is useful [30]. In this case, the microrobots can be regarded as the end-effectors of OT, which can carry out indirect manipulation tasks autonomously [30].

3. Visual Perception of Optical Microrobots

In this section, existing visual tracking techniques for micro-robotic systems are reviewed. The depth and pose estimation for optical microrobots using traditional methods and machine learning-based approaches are introduced.

3.1. Imaging and Tracking Techniques

The particle position detection system [31] can be used for position tracking of the micro-particles during optical manipulation. It has also been used for feedback control [32] and force measurements based on position fluctuations [33]. For most optical manipulation systems, the images of microrobots are obtained via optical microscopes and recorded by a high-speed charge-coupled device (CCD) camera.

In addition to using an optical microscope for imaging, magnetic imaging, X-ray, ultrasound imaging, or fluorescence imaging can be used for *in vivo* tracking of microrobots. The relative merits of different imaging modalities and the applications for tracking and monitoring microrobots have been reviewed [34].

A structural colors-based approach has been developed for tracking 3D-printed microrobots [35]. Different structural colors can be acquired by modifying the micropattern parameters, through which the images obtained using a microscope can be similar to fluorescence labeling but without the need of additional fabrication steps. Though the validation is conducted based on magnetic microrobots, the concepts can be applied to optical microrobots directly. Moreover, photoacoustic tomography has been used to capture optical microrobots in bovine or mouse blood. This imaging technique is effective for *in vivo* studies using optical microrobots [36]. Most of the optical microrobots are imaged by optical microscope for *in vitro* studies. Advanced imaging techniques for tracking microrobots for *in vivo* studies need to be further developed.

Algorithms have been developed for online monitoring and tracking of micro-spheres in OT-based systems [37]. The area of interest can be located via segmentation first, while the gradient information is obtained to determine the position of micro-spheres in the optical plane. Following that, signature curves are computed and utilized to estimate the position of the micro-spheres along the optical axis. Visual tracking of magnetic intraocular microrobots has also been developed by using model-based computer vision techniques [38]. However, these methods are difficult to be applied to tracking microrobots, especially those with complex shapes and are transparent. Machine learning-based methods have been investigated in recent years to track micro-objects. For example, a convolutional neural network has been used to track multiple particles and non-spherical objects such as bacteria under poor illumination conditions or in noisy environment [39]. In addition to tracking the 3D position of microrobots, 3D orientation estimation is also required for practical applications, thus requiring 6D pose estimation.

3.2. Vision Techniques for 6D Pose Estimation

In addition to tracking 2D planar motions of microrobots, vision-based 6D pose estimation techniques are important for micro-manipulation, which can support the closed-loop control of microrobots or autonomous micro-assembly tasks [40]. Moreover, it can improve the perception of the microrobots or target micro-objects in micro-scales, which ensures the ergonomics of 3D micromanipulation for the operators.

3.2.1. Traditional Methods

For microscopic image processing, traditional visual tracking methods can be used to determine the interaction forces between micro-manipulator probes and cells [41] when assisting manual cell injection, while the tracking fluorescent particles and molecules have been investigated based on vision techniques [42].

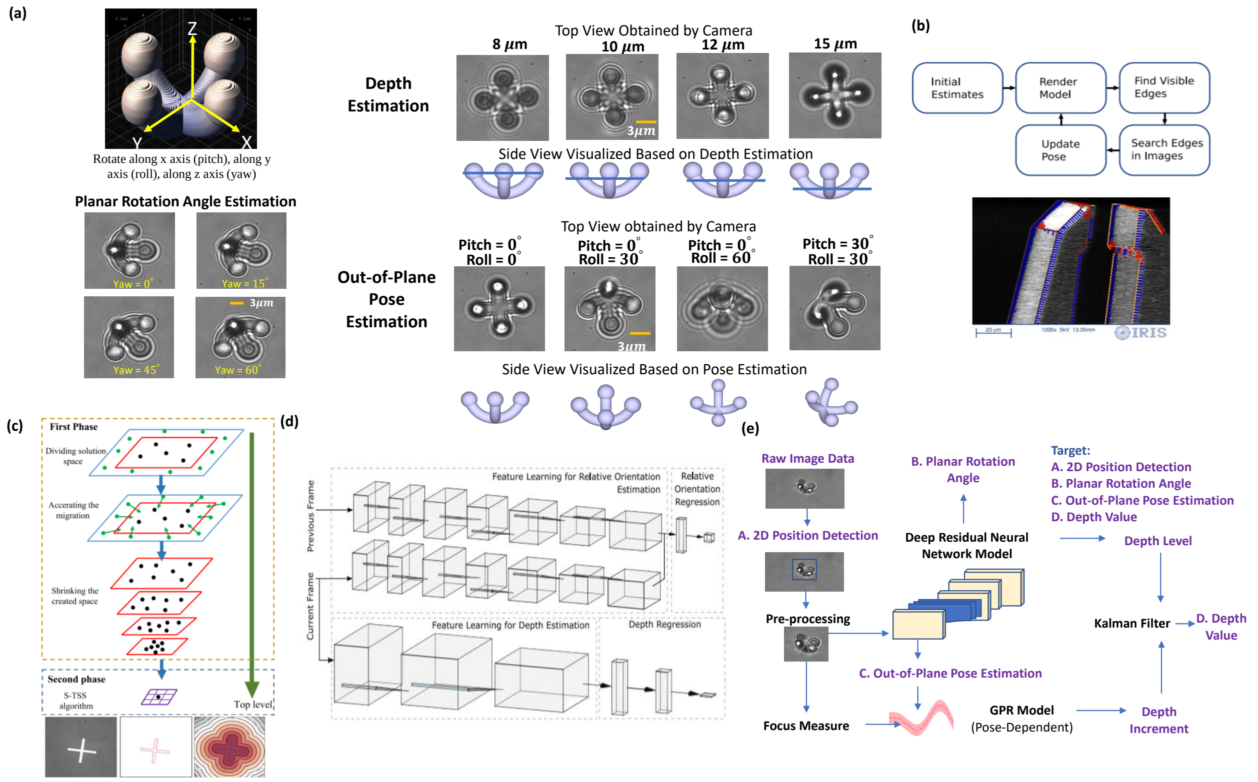


Figure 4: Example vision techniques used for the perception of optical microrobots. An example for (a) the definition of the coordinate and the illustration of the rotation angles for an optical microrobot; the illustration of the 6D pose estimation of the optical microrobot [51]. (b) An example of a feature-based method for tracking a microrobot under an electron scanning microscope [52]. (c) An example of using an enhanced microscopic vision-based approach to support high-precision measurement on planar motions of a micro stage [46]. (d) A CNN-based method for depth and 3D orientation estimation for optical microrobots [53]. (e) An architecture of 6 DoFs pose estimation for optical microrobot using a ResNet-GPR hybrid model[51]. Panels (a),(e) reproduced from [51] with permission. Copyright 2020, American Chemical Society. Panel (b) reproduced from [52] with permission. Copyright 2009, SAGE Journals. Panel (c) reproduced from [46] with permission. Copyright 2019, IEEE. Panel (d) reproduced from [53] with permission. Copyright 2019, IEEE.

Vision-based multi-DOF motion tracking has been widely explored in everyday macro-scale robotic systems. To build intelligent micro-scale robotic systems, vision techniques for tracking micro-objects should be explored. Vision-based tracking normally includes feature-based methods [43], template matching-based methods [44], statistical shape models, the phase correlation-based methods [45], and other multi-modal image processing methods [46].

Among all these traditional approaches, template matching is a simple technique that is commonly used. Two DoFs displacement measurements have been achieved with the accuracy in the nanoscale [47]. The position tracking of micro-objects has been investigated for optical trapping force estimation [48] and the characterization of biomolecular forces or soft tissues mechanical properties [49].

In micro-robotics, depth or orientation estimation for micrograppers, microrobots or carbon nanotubes (CNT) with Scanning Electron Microscope (SEM) has been investigated using feature-based vision techniques [50]. The visual tracking of intraocular microrobots actuated by the magnetic field has been implemented using model-based vision techniques [38]. However, more general approaches should be investigated for applications that require the manipulation of mobile microrobots with complex shapes where their poses keep changing and should be estimated.

3.2.2. Machine Learning-Based Methods

Compared to traditional approaches, machine learning-based algorithms, such as deep learning neural networks can provide more general solutions for 3D depth and pose estimation [54].

Fig. 4 (a) shows an example of depth estimation of an optical microrobot. It can be seen that the microscopic images of the microrobots obtained at different depth levels have large variations. By integrating convolutional neural networks (CNNs) and long short-term memory (LSTM) networks, the depth of optical microrobot can be estimated [55].

In addition to depth estimation, orientation estimation using CNNs based on a single microscopic image as observation has been investigated, where Fig. 4 (c) shows an example. CNNs have been employed for 3D pose estimation from a single image [56]. Simultaneous depth and 3D orientation estimation of optical microrobots can be achieved via CNNs as well [53] (see Fig. 4 (b)). However, the model training process is time-consuming while a large database should be collected to enable the effective training of CNNs.

Moreover, the pose estimation was implemented with a relative orientation estimation mode, which means that accumulative errors may affect the accuracy of pose estimation. Therefore, future research should focus on learning from a small database with data-efficient approaches [57]. More recently, a ResNet-GPR hybrid model has been proposed, which can enable precise 6 DoFs pose estimation of optical microrobots (see Fig. 4 (d)). Domain adaptation has also been verified that the proposed method can be generalized to microrobots with different structural designs.

4. Manipulation of Optical Microrobots

In this section, the state-of-the-art research for the implementation of indirect manipulation via OT is investigated. Potential solutions for enabling out-of-plane rotation and dexterous 6 DoFs manipulation via OT are explored, including sculpting the light field and shaping the structures of the microrobots.

4.1. Optical Microrobots for Indirect Manipulation

To conduct non-invasive manipulation on cells [29], dielectric micro-beads with reasonable size can be used as end-effectors of OT [30] to extend the manipulation ability of OT. They can help reduce the exposure of the samples to the laser and aid studies of manipulating laser-sensitive samples when using OT in biological studies. We address in this section mainly the manipulation of optical microrobot with complex shapes as the literature of indirect manipulation of micro-beads is already covered in previous reviews [58].

4.1.1. Micro-Fabrication

Emerging micro-fabrication techniques have contributed to the fabrication of optical microrobots with increasingly sophisticated shapes to enable dexterous indirect optical trapping and optical manipulation, induced convection [59], thus facilitating the deployment of general solutions for biomedical engineering [60].

3D micro-printing technologies, such as Two-Photon Polymerization (TPP), is one of the attractive tools for microrobot fabrication [61]. With this technique, microrobots with spherical structures as the optical trapping handles can be fabricated. Examples of optical microrobots in such settings are shown in Fig. 5, some of which have been used as new functional optical micro-robotic tools for biological experiments.

In addition to TPP, soft lithography has been used for micro/nano-scale patterning of organics/polymers [62] or other soft matter. Electron beam etching, photolithography and other technologies can be used to support complementary micro-fabrication processes for constructing micro-scale components from other types of materials.

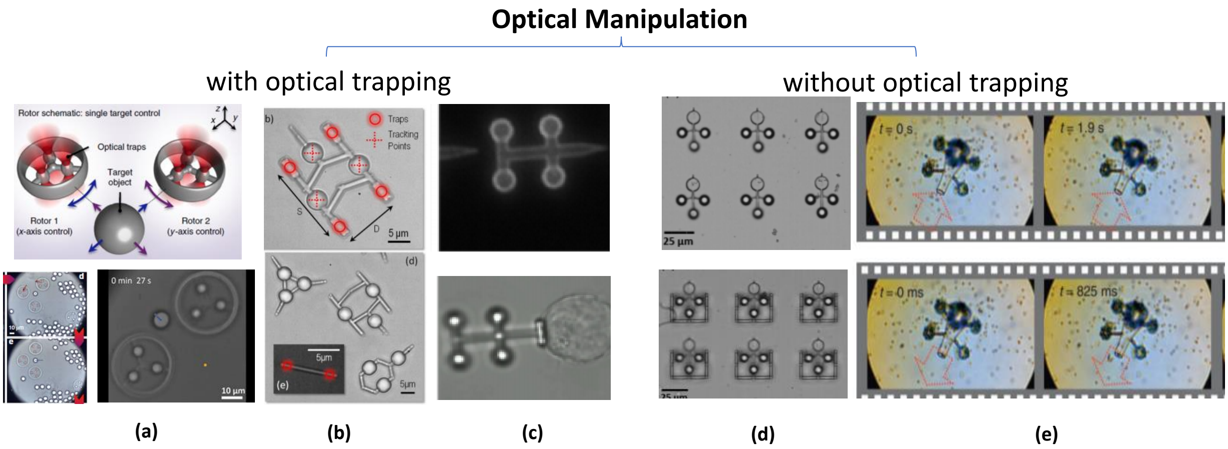


Figure 5: Examples of microrobots actuated by laser beam including with and without optical trapping. (a) The design of the microrobots with the shape of rotors and the experimental verification of the indirect manipulation via OT with hydrodynamic manipulation [63]. (b) Ultralow force surface scanning probe developed for the characterization of sensitive samples [64]. (c) Microtools functionalized with fluorescent streptavidin [65]. (d) Microrobot equipped with a thermoplasmonic disk for mixing in microfluidic channels. [66]. (e) Microrobot with a hollow-body syringe-like structure are actuated via thermoplasmonic-induced convection [59]. Panel (a) reproduced from [63] with permission. Copyright 2019, Springer Nature. Panel (b) reproduced from [64] under the terms of the CC-BY license. Copyright 2012, The Authors, published by Optica Publishing Group. Panel (c) reproduced from [65] with permission. Copyright 2012, Elsevier. Panel (d) reproduced from [66] under the terms of the CC-BY license. Copyright 2018, The Authors, published by Optica Publishing Group. Panel (e) reproduced from [59] with permission. Copyright 2016, Nature Publishing Group.

Optical microrobots with complex shapes can expand the applicability of optical manipulation. Therefore, advanced micro-fabrication techniques are worth developing to increase the resolution of micro-printing and the fabrication speed.

4.1.2. Optical Manipulation With Optical Traps

With increased precision and dexterity, optical microrobots can assist tasks such as cell enucleation and polar-body biopsy. It can be used for imaging at microscale as a scanning probe [67]. It can also be used as a probe to measure force after calibration while taking shape-dependent thermal fluctuations into consideration [68].

Most of the microrobots are designed to have spherical handles attached to their body, which can ease the trapping. For example, Fig. 5 (a) indicates a novel optical microrobot was developed and served as a micro-motor to create a new mechanism of micro-manipulation based on near-field hydrodynamics. However, the control is limited to planar motion. Fig. 5 (b) shows an example when a microrobot is used as an optical scanning probe [64]. Fig. 5 (c) shows a microrobot with surfaces coated with protein streptavidin and gold nanoparticles and equipped with four spherical handles for ease of optical manipulation [65].

All the aforementioned studies have verified the effectiveness of using optical microrobots to implement indirect manipulation via OT based on optical trapping. With optical microrobots for indirect manipulation [69], the laser-induced damage can be avoided in biological research. Moreover, transporting objects that have a relatively large size can be achieved.

4.1.3. Optical Manipulation Without Optical Traps

In practice, the force involved in optical manipulation includes gradient and scattering forces. When the gradient force is larger than the scattering force, stable trapping can be achieved. Without optical trapping, manipulation is mainly through the exchange of angular momentum with light. For example, polarization-controlled multiple-beam OT can be used to control birefringent particles to rotate [70]. Moreover, optical pulling has been explored, which is implemented by a Bessel beam [71], which is composed of near-glancing plane-wave components.

In certain conditions, light can pull particles that favor forward scattering [72], which has been depicted as 'tractor beams' as in popular science fiction [73]. Thermal effects can also be used in OT for actuation. For example, a "micro-syringe" has been created. Thermally-induced flow can be created in response to the laser, since a thin metal layer is developed inside the 3D printed chamber. Fig. 5 (d) shows that cargos can be loaded and unloaded using photothermally induced convection currents from micro-robots, which have thin metal layers inside their bodies to enable the conversion of incident optical energy into heat. In this case, OT is not only used for positioning, but the local heating created by the laser at a small metal spot within the microrobot's liquid-filled body can be used to trap or release a payload [59]. A disk-like feature was incorporated (Fig. 5 (e)) and the robot was further functionalized by electron-beam deposition. Upon illumination from a laser beam, the gold layer on the disk creates off-resonant plasmonic heating, thus generating thermoplasmonic-induced natural convection.

4.2. 6D Manipulation of Microrobot

Optical force can be used to replace the actuation mechanism in traditional robotic systems [74]. To extend the manipulation capabilities of optical microrobots into broader applications, customized structure design, patterns of light and advanced control strategies are used to enable 6 DoF manipulation of the target objects. Currently, planar manipulation can be easily achieved while out-of-plane rotation is relatively difficult for conventional OT as its optical traps are constrained on the same focal plane.

Rod-shaped bacterial cells can be rotated by an oscillating linear optical trap [77]. To enable the microrobot to rotate, optical forces that are displaced from the axis of rotation can be used to generate toques. The orbital angular momentum (OAM) of light [78] can be used to rotate micro-objects using angular momentum exchange. However, ongoing researches are worth exploring for stable indirect out-of-plane manipulation in biomedical applications.

As optical trapping is effectively through light-matter interaction, the orientation of optical microrobots can be controlled by regulating the wavefront of light, altering the optical path, or designing microrobots with the articulated structure or customized shapes. The hybrid control method, which combines the advantages of different physical fields for actuation can assist the orientation control. To implement 6D manipulation for indirect cell control, sculpting light field and tailoring the structure of the microrobots are two main approaches, and will be detailed as follows.

4.2.1. Sculpting Light Field

Sculpting the light field is one of the main research directions for improving the manipulation abilities of OT. 3D SLM can be used to control the position of optical traps independently for out-of-plane manipulation [79]. This has been used to manipulate lipid nanotubes in fluidic environments. A pair of galvanometer-mounted mirrors and an SLM are used to construct the optical manipulation system [75]. The microrobots designed for 6D manipulation and the control mechanism using three or four laser spots are illustrated in Fig. 6 (a). However, this technique is not widely employed due to technical complexity and the high cost.

Beam-shaping can be used to generate multiple traps simultaneously. With advanced laser modes, optical trapping can be realized by ensuring the efficient use of light power. Combined with the real-time reconfiguration of the beams, scattering, and absorption of light by the samples can be eliminated to some extent.

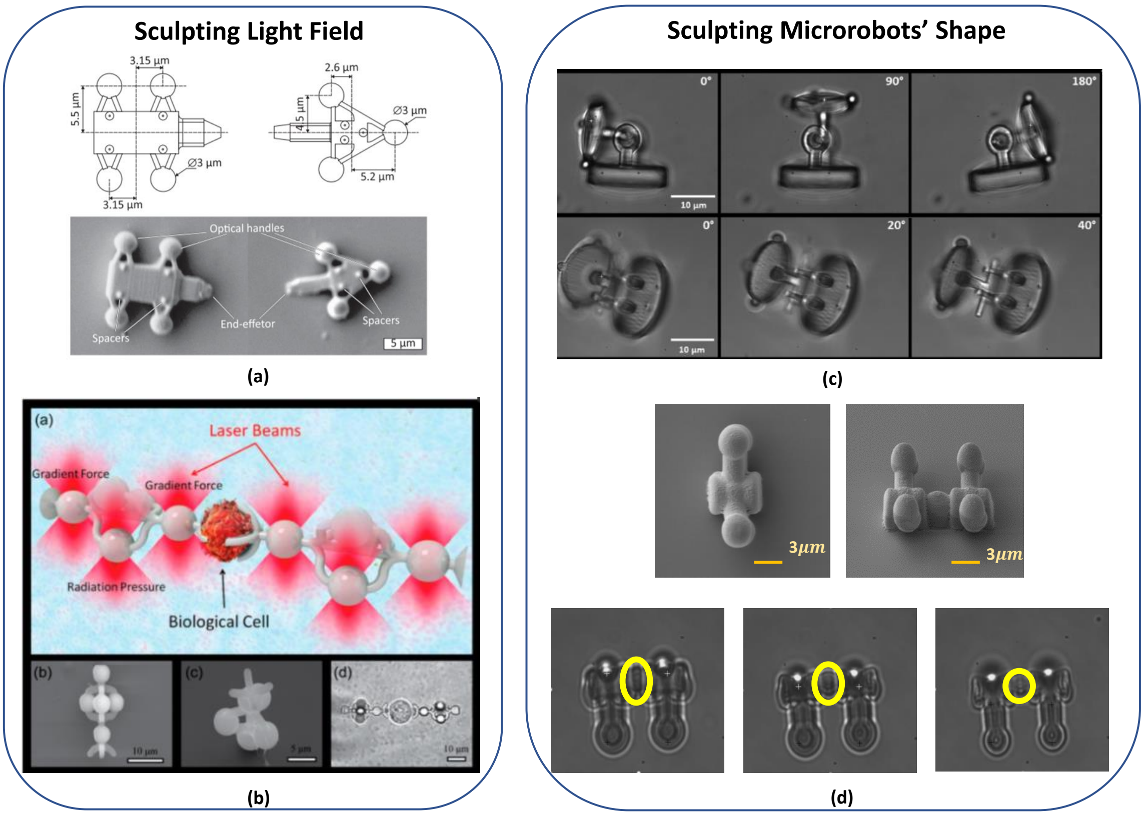


Figure 6: Sculpting the shape of the microrobots for the implementation of 6D manipulation actuated by conventional OT. (a) Illustration of 6-DoF real-time control of optical robots based on high-bandwidth 3-D actuation via multi-traps [75]. (b) The sketch and SEM image of microrobots with fork-like clamps actuated by HOT for indirect cell manipulation [76]. (c) Optical manipulation of the mobile articulated microrobot (Image obtained from [12]). (d) Two microrobots are used to rotate a micro-object for observation in cooperative manner [14]. Panel (a) reproduced from [75] with permission. Copyright 2019, IEEE. Panel (b) reproduced from [76] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Multidisciplinary Digital Publishing Institute. Panel (c) reproduced from [12] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Wiley. Panel (d) reproduced from [14] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Wiley.

Wavefront shaping can be implemented by displaying desired holograms on diffraction optical elements [80], such that multiple optical traps in 3D can be created simultaneously. The effect of scattering and light focusing *in vivo* has been investigated [81].

Wave-guided optical waveguides [82] have been developed recently, through which micro-objects can be optically controlled into any arbitrary orientation. Tomographic active optical trapping of arbitrarily shaped objects by exploiting 3D refractive index maps has been investigated in [83]. A computer-generated hologram [84] can be dynamically reconfigured in real-time. It is commonly used to split the laser beam into multiple components with complete control over the focal positions of the individual beams. This multiplexing capability enables concurrent manipulation of multiple microrobots or micro-objects.

HOT can be used to actuate microrobots independently using multiple focused laser beams. By changing the depth of different laser spots independently [85], out-of-plane rotational control can be achieved. For example, Fig. 6 (b) illustrates an optomechanically-driven microrobot with full-3D manipulation capabilities. It can rotate a living cell in the out-of-plane direction actuated by HOT, clamped between a pair of fork-like structures in a pure liquid.

However, the complex and bulky setup of HOT limits its wide applications. Moreover, it is more costly compared to conventional OTs [86]. It may be challenging to transfer the 6D manipulation framework from HOT to conventional OT to rotate a micro-object for observation in cooperative manner.

Sculpting the shape or other properties of microrobot can improve its optical manipulation capability to accomplish specific tasks. It is therefore necessary to develop more general methods for out-of-plane control.

4.2.2. Sculpting Microrobots' Shapes

Sculpting the shapes of microrobots can be another promising solution for the implementation of 6D manipulation of microrobots [87]. The trapping force distribution for a microrobot under OT is normally influenced by the features of the target object. The structure design for microrobots has not been fully studied, which serves as one potential solution to realize out-of-plane control via planar multi-spot OT. To this end, it is worth investigating the shapes of microrobots, and verifying the concept of out-of-plane control by altering the force distribution through the interaction of the microrobot and the optical beam to implement 6D control via OT.

Based on the basic principles of OT, most of the target objects for trapping are micro-spheres of different sizes and refractive indices. Nowadays, complex structures can be fabricated for optical manipulation [88], thanks to the advances in microfabrication techniques. Sculpting the shape or other properties of microrobot can improve its optical manipulation capability to accomplish specific tasks. Therefore, the optimization of topology and structure can be considered, while the modification of microrobots' surface materials may also be helpful for biomedical applications.

With the TPP based micro 3D printing techniques, the fabrication of articulated microrobots has become flexible. To enable indirect orientation-based control via planar OT, an articulated robot has been fabricated for dexterous cell handling [12], as shown in Fig. 6 (c), which can transform planar motion to out-of-plane rotation [12], although the control relationship between the position of the laser spots and the rotation angle in the out-of-plane direction hasn't been fully explored. The micro-fabrication errors may pose challenges to repeatable and stable control of microrobots in 3D.

Moreover, the dominance of adhesion forces is normally the barrier for using articulated microrobots in biomedical research, since the joints and links or other discrete moving parts may be bonded to each other due to Van der Waals forces [89]. To further develop an articulated optical robot, mechanical decoupling of different components of the robots should be considered, while the shapes of each component should be carefully designed to enable dexterous operation.

Micro-objects with spherical shapes are known as the easiest objects to trap using single Gaussian beams. When the refractive index of the micro-sphere is higher than that of the surrounding medium, they can be strongly drawn into the optical trap than micro-objects with relatively flat surfaces. The reason is that spherical objects are isotropic and the scattering force generated by the laser beam during the interaction with the micro-objects is much lower compared to flatter micro-objects. As for the situation where the refractive index of the micro-object is lower than the surrounding medium, doughnut-shaped objects can be trapped more easily and stably by using a Gaussian beam [90].

For cylindrical micro-objects, experiments of trapping cylindrical viruses have shown that they tend to orient themselves with the axis of the optical trap [91]. To enable the optical trapping of micro-objects with a cylindrical shape, spherical features are added to the main body of the microrobots to enable effective trapping [92]. However, out-of-plane rotation is difficult to achieve.

That is to say, except for adding spherical structures to the body of microrobots, anisotropic shapes can be considered as the structure of microrobots. For example, a micro-motor can be developed by making use of the radiation pressure to generate the out-of-plane rotation motions [93]. To rotate a micro-motor, light can be used to push the rotating part of a microrobot as a result of the scattering force [94], which forms an optical "paddle wheel".

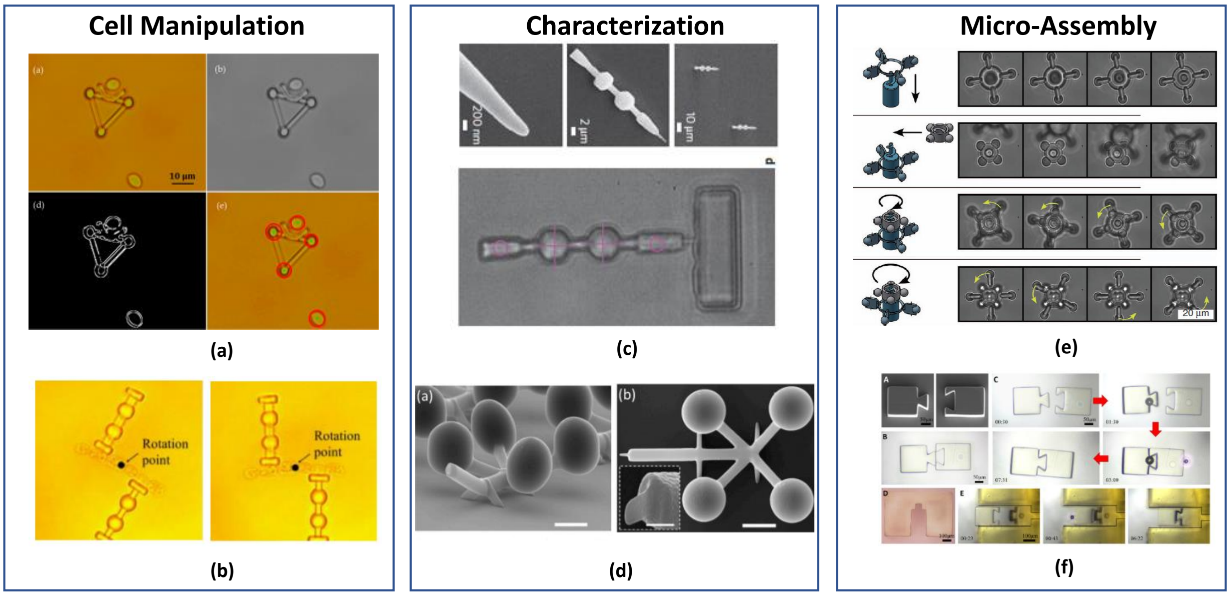


Figure 7: Examples of using optical microrobots for various applications. (a) Cell manipulation using microrobot with spherical handles for optical trapping [96]. (b) Multiple microrobots are actuated by HOT for translational and rotational manipulation of filamentous cells in cooperative manner [97]. (c) A microrobot with tapered cylindrical shape, fabricated by TPP, has been designed as a passive force clamp [67]. (d) A microrobot was designed to probe the endothelial cells grown on vertical polymer walls for cell elasticity measurement [98]. (e) Nut-like microrobot, which can be manipulated by optical traps for micro-assembly with a fixed screw [99]. (f) Assembly of two microparts with dovetails [100]. Panel (a) reproduced from [96] under the terms of the CC-BY license. Copyright 2019, The Authors, published by Multidisciplinary Digital Publishing Institute. Panel (b) reproduced from [97] under the terms of the CC-BY license. Copyright 2019, The Authors, published by Optica Publishing Group. Panel (c) reproduced from [67] with permission. Copyright 2014, Nature Publishing Group. Panel (d) reproduced from [98] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Multidisciplinary Digital Publishing Institute. Panel (e) reproduced from [99] with permission. Copyright 2017, Nature Publishing Group. Panel (f) reproduced from [100] with permission. Copyright 2019, Wiley.

Rotor-like micro-fabricated particles have been proven to be able to rotate in a regular Gaussian beam optical trap along an axis perpendicular to the laser beam axis [95]. Though the angular velocity can be generated for the micromotors mentioned above, the desired out-of-plane pose of the micromotor cannot be controlled. To realize stable out-of-plane pose control via OT for microrobot, a new design for microrobot structure should be further explored.

By controlling the positions of multiple laser spots and altering the generated force distribution of an optical microrobot with a special shape, out-of-plane rotation can be implemented. Fig. 6 (d) shows the concept of out-of-plane rotation of microrobots via a multi-spot laser beam. By adjusting the distance between two laser spots, the out-of-plane pose control of the microrobot using conventional OT systems can be implemented. The shapes of the microrobots and the control strategies for the implementation of dexterous 6 DoFs manipulation should be further explored. The optimal design of the microrobot can be applied to different applications, with automation techniques incorporated to enable closed-loop control.

5. Biomedical Applications

To date, optical microrobots actuated by OT have been successful used in applications including pumping [106], characterizing mechanical properties of cells [107], scanning surface [67], directing the growth of tissue [108], and transporting target objects [63]. They are playing an increasingly important role in the post-CRISPR life-science research landscape, where single-cell isolation, clonal expansion, and RNA sequencing are required. Several typical

Table 1: Optical Micro-Robotics for Biomedical Application and Associated Challenges.

Category	Applications	Challenges	Ref
Cell Manipulation	Cell transportation	Microrobot Design; Biocompatibility	[101]
	Assist cell surgery	Out-of-Plane Manipulation	[102]
Biological Characterization	Measure force properties	Microrobot structure design and calibration	[103]
	Measure environmental properties	Materials and fabrication; Calibration	[104]
	Scan surface	Microrobot control; Force characterization	[67]
Micro-Assembly	Construct complex micro-structure	Perception and control of microrobots	[99]
	Construct vascular-like micro-channel	Microrobot parallel control; Biocompatibility	[105]

applications such as cell manipulation, characterization, and micro-assembly will be introduced in this section. We summarize the applications and the associated challenges in Table. 1.

5.1. Cell Manipulation

Different types of target cells can be trapped by optical field and transported to the target locations [109]. Due to the challenges of optical micro-manipulation, many efforts related to visual tracking, path planning and control have been made to enhance the precision for cell sorting using OT. For the transportation of cells, stochastic path planning [110], modified A* path planning [101], have been developed, which aims to eliminate force disturbances caused by blood flow inside the tissue.

OT can also be used to assist cell surgery, for example, it has been used to manipulate an optically transparent microrobot with a carbon nanotube probe for cell puncture [102]. A microrobot has been designed with spherical handles that can be trapped and moved by OT and a V-shaped head for pushing cells of different sizes (see Fig. 7 (a)). Fig. 7 (b) shows that two microrobots can be actuated by HOT for translational and rotational manipulation of filamentous cells in cooperative manner [97]. This capability enables potential applications for single-cell-based assays.

5.2. Biological Characterization

Polystyrene micro-beads can be attached to macromolecules and trapped for force measurement or mechanical properties of the molecules [33]. To investigate the mechanical response of the cardiomyocyte cell, two micro-beads were attached to a cell and manipulated by optical traps during cell stretching [103]. To measure the temperature change of virus-infected cells, a microrobot with temperature sensing is controlled by HOT [104]. Gel-microbeads, connected to circular shape, have been manipulated by OT to measure local pH value around single yeast cell [111].

Optical microrobots have been used as tools to measure force at microscale after proper calibration considering shape-dependent thermal fluctuations [112]. An optically trapped cigar shaped probe has been used as a probe to scan the surfaces of biological particles, forming a novel imaging technique [113].

As shown in Fig. 7 (c), a tapered cylindrical microrobot fabricated by TTP has been used to clamp micro-objects [67]. Fig. 7(D) illustrates a microrobot designed to probe the endothelial cells grown on vertical polymer walls for cell elasticity measurement. However, the characterization is limited to probing in a lateral direction.

5.3. Micro-Assembly

Although there are many existing techniques that can be used for micro-fabrication of 3D micro-optical devices, most of them can only deal with a single type of material. Since OT is capable of manipulating micro-objects with varying material properties in a non-contact and biologically friendly manner, it can be used as a promising tool for micro-assembly of microtools with multiple materials [114].

Optical microrobots have been used for building blocks for optical micro-assembly systems [115]. For example, Fig. 7 (e) indicates that an optical microrobot has been served as the optical screw-wrench for micro-assembly [99]. It was used for the nut-and-screw assembly at the microscale by optical manipulation [99], where a screw was fixed on a plane surface. As for the nut, it has four spherical handles that can be optically trapped and transported to assembly with the fixed screw. Fig. 7 (f) indicates another example of micro-assembly for complex structures using optical force. Through laser-induced heating effect, the bubbles in saline solution can be controlled to move, fix, lift, and drop microparts for micro-assembly of complex structure [100].

The development of real-time optical trapping with multiple laser beams has enabled the development of optical assembly workstations [116]. With this technique, a large number of micro-beads can be assembled in 3D [117]. By adding spherical handles onto micro rods, they can be controlled to build reconfigurable structures [118]. Micro-assembly of a vascular-like microchannel has been implemented [105], which was implemented by a group of microrobots in a cooperative manner.

Cellular development has a close relationship with the surrounding micro-environment [119]. An optical micro-assembly platform has been developed for reconfigurable micro-environment control in biomedical studies [118]. With this technique, cellular development or reconfigurable bio-compatible scaffolds for 3D tissue engineering can be implemented using feature-rich microtools actuated by multiple, real-time configurable counterpropagating-beam traps. Moreover, optical binding or shaped laser beams [120] can be used for self-assembly.

6. Future Roadmap

To bring further benefits of combining optical manipulation with microrobots for biological applications, many future research directions need to be explored. These include the development of versatile optical robotic systems, novel manipulation mechanisms, and advanced control systems. In addition, issues related to new materials, actuation schemes, sensing mechanisms, and novel fabrication methods need to be further explored.

6.1. Towards Versatile Optical Robotic Systems

To further enhance the capabilities of optical microrobots for dexterous manipulation, advanced material, optimal structure design, and fabrication technologies should be considered.

6.1.1. New Materials

For optimal design of microrobots manipulated by OT, the main target is to enlarge the range of achievable forces in optical manipulation. One potential method is to utilize antireflection-coated high-index materials [121] for optical trapping handles, which can better convert incident optical momentum flux to usable optical trapping force to the nanonewton level.

Biocompatibility is also an important aspect to consider particularly for *in vivo* applications. Biocompatible polymers that enable effective optical trapping should be developed. For example, some particular liquid crystalline networks (LCN) have demonstrated biocompatibility by showing the ability to support the maturation of cells in cultures [122].

The current materials used for fabricating the micro-robots include commercial photoresists such as IP-L,[59] IP-G,[67]. Other materials that have been reported include the SU-8, [123], Femtobond 4B,[99], NOA63[124]. For example, SU-8-based microrobots with streptavidin coatin have been attached to a cell for indirect micromanipulation of single live cells [125]. Some light-responsive materials can be incorporated into the microrobots, which can provide embedded intelligence to the microrobots with enhanced dexterous manipulation capabilities. Moreover, the microrobots should be functionalized to carry and release drugs, which is fundamental for targeted drug delivery or therapy.

6.1.2. Structures

The properties of optical manipulation can be influenced by the properties of optical fields as well as the microrobots' structure. Therefore, another promising approach to increase the optical trapping force is by topological optimization [126].

The optimal structure design for microrobot can benefit research of optical lift by finding the reasonably optimal "lightfoil" shape for predetermined light illumination patterns. Moreover, computational optimization techniques can be used to assist the selection of optimal structural topology for microrobots to adapt to specific applications. To reduce the time for experiments, advanced simulation with accurate modeling of the dynamics for controlling microrobots should be developed, which eases the design of microrobot and enhance the efficiency for relevant research.

6.1.3. Fabrication Schemes

As for micro-fabrication, scalability should be considered. The time for microrobot fabrication increases significantly when multiple microrobots are required to be fabricated. The resolution and precision of the fabrication can be further enhanced when developing advanced micro-fabrication methods. In addition, microrobots can be functionalized with advanced post-processing methods after micro-fabrication to realize their full potential for micro-manipulation tasks. Incorporating effective functionalization techniques can endow microrobots with sensing possibilities [127].

6.2. Towards Manipulation with Advanced Control System

6.2.1. Ergonomic Control interfaces

An optimal control interface for micro-manipulation is a prerequisite for many biological applications. This can ensure ergonomics for human operators when they access the microscale domain for specific tasks. Therefore, the design of intuitive control interfaces for operating micro-robots should be considered, which may stimulate further progress in micro-robotic research [128].

A human-robot interface based on gaze contingent control has been developed to facilitate micro-assembly tasks [129]. With this framework, the operator's intentions can be recognized, while the optical traps can be interactively reconfigured based on the recognized operator's eye fixation point. In addition, haptic constraints can be generated based on the user's eye gaze to control the position of the micro-objects for micro-assembly. Force-sensing combined with Virtual Reality visualization can provide users with intuitive control when performing complex tasks [130].

6.2.2. Parallel Control

Multi-robot cooperative control is a promising research direction. Collective actuation of multiple microrobots can boost the throughput in clinical applications compared to operating a single microrobot.

To increase the operation efficiency during optical manipulation experiments, multiple microrobots can be controlled by multiple optical traps simultaneously. For example, the control of opto-thermocapillary flow-addressed bubble microrobots has been developed for high-throughput micro-assembly of micro-objects [131]. Advanced control strategies like iterative algorithms [132] and reinforcement learning [133] can be useful for multi-robot selective actuation. This provides opportunity for OT to actuate autonomous optical microrobots for a controlled collective swarm manipulation [134].

At the macroscale, sensors and signal processors can be integrated with the robots for communication with ease. However, at the microscale, the system integration of these additional components to the tiny body of the microrobots is challenging. To enable multiple optical microrobot cooperative control, miniaturization of electronic components should be further developed. Moreover, on-board power could be investigated for optical microrobots using conductive polymers to build the surface electrochemical actuator.

6.2.3. Closed-Loop Control

In recent years, optical micro-robotics have sparked significant excitements, where the proposed microrobots have capabilities of sensing, control and even decision-making. Thus far, most of the microrobots are manipulated via moving the optical traps manually. However, this is a tedious process with low efficiency. Precise and automatic control of microrobots with a fine tool has been investigated for operations at micro-scale [135]. Microrobot actuated by OT automatically for indirect manipulation has been studied [136]. Therefore, it's worthwhile to implement automatic manipulation of biological cells via optical microrobots actuated by OT [137]. This can enable optical manipulation to become an effective tool for non-experts and improve the repeatability of specific applications in the future.

The ultimate goal is to develop an intelligent optical microrobot system for dexterous cell manipulation, which can move and manipulate the target micro-objects autonomously. This requires adapting the micro-robotic design, perception, planning, and control techniques that have been well-developed from macro-scale to micro-scale and address the challenges specific in micro-scale domain [30].

6.3. Towards In Vivo Applications

One of the significant challenges for optical manipulation is the process of transferring these techniques from laboratories to *in vivo* studies [140]. Laboratory-based optical manipulation techniques are getting increasingly mature. Issues related to cost-effectiveness, ergonomics, and other practical factors should also be taken into account in the technical translation for *in vivo* applications.

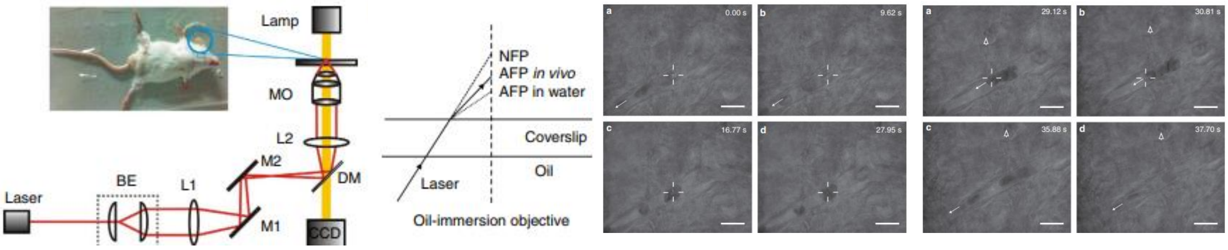
6.3.1. In Vivo Optical Trapping

Optical microrobots could be useful for microsurgery in peripheral tissues (such as skin), where focused light can penetrate through to manipulate. Trapping red blood cells (RBCs) within living mice in 3D for *in vivo* study using OT has been demonstrated [138], which can produce or eliminate thrombosis (see 8 (a)). Moreover, optical manipulation of nanoparticles and cells inside living zebrafish has been investigated, through which the feasibility of using OT to trap nanoparticles adhered on cells *in vivo* has been verified [141].

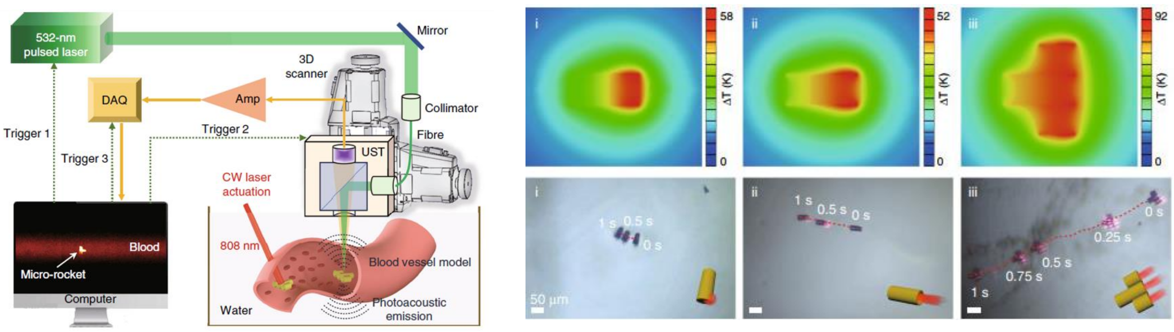
A disturbance compensation controller has been developed to counteract the effect of hemodynamic forces acting on cells when manipulating them using OT [142].

6.3.2. In Vivo Actuation of Microrobots

All the work mentioned above is related to direct manipulation using OT for *in vivo* applications. As for indirect manipulation using light, a novel microrobot with the shape of a rocket has been proposed, while an all-optic driving and imaging system was incorporated into the micromanipulation system to track microrobots in blood with



(a)



(b)

Figure 8: Examples of using optical manipulation for *in vivo* applications. (a) Experimental setup for *in vivo* optical trapping of RBCs using OT [138]. (b) Schematic illustration, simulation and experiment results of actuation and tracking of microrobots with rocket-like shape in the blood. Image obtained from [36]. Panel (a) reproduced from [138] under the terms of the CC-BY license. Copyright 2013, Nature Publishing Group. Panel (b) reproduced from [139] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Nature Publishing Group.

microscale resolution [36], as shown in Fig. 8 (b). The microrobot has a shape of a rocket-like triple tube structure, which can enable the microrobot to achieve quick motion control under weak driving light. With this structure, the microrobot can be driven in viscous media at high speed and can be observed when it enters the bloodstream.

6.3.3. Integration with Imaging Techniques

Integration of optical traps with imaging modalities is an important step towards precision biomedicine and the construction of the feedback loop mechanism for various applications that require assistance from optical microrobots.

Fluorescence imaging has been used for tracking magnetic microrobots *In vivo* [143]. Moreover, a photoacoustic imaging technique has been developed for tracing magnesium propelled motors located at a mouse intestine [144]. Ultrasound imaging has been used to detect bubbles generated by a chemically driven microrobot or to track soft and resilient magnetic cell microrobots [145]. Though the applications mentioned above were not used for tracking optical microrobots, the technologies can be transferred and integrated with optical manipulation system.

6.3.4. Operation in Cell Culture Media

To take full advantage of the optical manipulation techniques for *in vivo* studies, the optical microrobots should be able to perform the experiments in aqueous solutions and even in living organisms. However, efficient optical manipulation of microrobots in a real biological environment is challenging due to the inherent properties of light-object interactions (e.g., hydrogen bonding), optical properties that influence the trapping forces (e.g., refractive index, scattering properties, turbidity), microrheological properties (e.g. viscoelasticity), and the high variability of biological samples [146]. For example, optical trapping in a complex biological fluid such as mucus is challenging, due to the poor visualization and nonspecific binding. Therefore, optical trapping experiments in biological environments or complex media, are necessary for the evaluation of optical manipulation techniques.

Real-time phase manipulation has been investigated to overcome the challenges mentioned above. For example, optical phase conjugation [147], wavefront correction algorithms[148] can be used. To confer stealth, antifouling or nonimmunogenic properties, the material and the chemical properties of the microrobots should be tailored while post-processing of microrobots after fabrication should be designed properly [149]. Moreover, the use of metasurfaces is another research direction to improve light focusing in turbid media [150].

6.3.5. Optical Fibre Tweezer

In addition to utilizing the thermal effects of light to actuate microrobots, optical fibre can be envisioned for potential *in vivo* micro-manipulation tasks. Fiber-based traps can create multiple 3D traps for simultaneous manipulation of micro-objects [151]. With aberration corrections [152], optimized trapping of target objects at ultralow powers can be achieved during optical manipulation. Optofluidic devices can also be created for creating optical traps by integrating the 3D structure with built-in micro-optical elements [153]. This can eliminate the need for bulky microscope objectives or external beam modulation. More details can be found in [154], which can inspire the optical manipulation techniques for *in vivo* applications.

6.4. Discussions

Despite the versatility of optical manipulation, one drawback of OT is that most electromagnetic energy in the visible range is blocked by common obstacles. Therefore, a direct line-of-sight is usually required to operate light-powered devices. For *in vivo* applications this can prove difficult, unless an optical fibre or fibre bundle can be incorporated into the system.

To summarize, optical microrobots represent an attractive research area of robotics for biomedical engineering. Among all the relevant techniques, smart materials have had a significantly profound impact on the development of optical microrobots. Supported by the latest advances in materials as well as 3D micro-printing, microrobots with complex structures can be fabricated with good reproducibility and yield, further expanding the use of OT for micro-object manipulation. To enable the precise manipulation of micro-objects or cells, several key aspects are required to be considered for building up the micromanipulation system. These include the 6 DoFs manipulation, as well as accurate perception of the microrobot's pose and visual feedback for the operator to conduct a manual operation or serve as the fundamental functions for the implementation of closed-loop control. Whilst most of the available technologies are still at a laboratory development stage, they represent a promising future for applications, including cell manipulation, characterization, and micro-assembly.

7. Conclusions

In this review, we provide an overview of optical trapping, manipulation based on OT for indirect manipulation of microrobots, which outlines the way forward for the development of intelligent optical robotic systems. For optical manipulation, we focus on recent technical advances of dexterous out-of-plane manipulation via OT through sculping light fields or shaping the microrobots. As for the vision and control of microrobots, existing methods and data-driven methods are investigated, while the future roadmap for developing optical manipulation for biomedical applications is illustrated. In this review, we have highlighted the applications and perspectives of optical manipulation of microrobots in biomedical applications. We have further discussed the techniques for constructing single and multiple optical traps based on alternating laser modes or conducting beam-shaping.

Although optical manipulation techniques are promising tools for a wide range of applications, further developments are necessary. These include, for example, new microfabrication methods for versatile optical microrobots with different materials and functions, robust perception and dexterous manipulation via novel optical systems, and effective measures for enduring biocompatibility for *in vivo* applications. We envision that with improved design of both optical manipulation systems and microrobots, the optical robots can act as "micro-surgeons" to perform cell-based interventions, bringing tangible benefits to biomedicine then helping shape the future of healthcare research.

ACKNOWLEDGMENTS

D.Zhang would like to acknowledge Royal Society Research Grant (RGS\R1\221122). This Work was performed at Imperial College London, Shanghai Jiao Tong University and University of Bristol and Bristol Robotics Lab.

AUTHOR CONTRIBUTIONS

Conceptualization and writing, D.Z, G-Z.Yang. All authors contributed to the proof-reading, revision and valuable discussion of the manuscript.

Declaration of Interests

All authors declare that they have no conflicts of interest.

Figure Legends

1: Illustration of the scope of the review: OT based indirect manipulation of micro-objects using optical microrobots, while the main focus of this review lies in the perception and manipulation of optical robots for biomedical applications.

2: Number of publications and citations containing the terms "optical tweezer," "micro-robotics," "micromanipulation," "optically-driven," "optically-control," "light-powered," and "microrobot" from January 1980 to the December 15, 2021, according to Google Scholar.

3: OT system and a schematic diagram of optics, and the concept illustration of optical microrobot manipulation via different OT setups. (a) The conventional OT system which can generate multiple laser spots based on time-sharing; the schematic diagram of optics for the corresponding OT system. (b) The concept illustration of dexterous manipulation of optical microrobots using conventional OT systems. (c) The conventional holographic optical tweezer (HOT) system which can generate multiple laser spots; the schematic diagram of optics for the corresponding HOT system. Image obtained from [13]. (d) The concept illustration of dexterous manipulation of optical microrobots using HOT system. Panel (a) reproduced from [14] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Wiley. Panel (c) reproduced from [13] with permission. Copyright 2019, IEEE.

4: Example vision techniques used for the perception of optical microrobots. An example for (a) the definition of the coordinate and the illustration of the rotation angles for an optical microrobot; the illustration of the 6D pose estimation of the optical microrobot [51]. (b) An example of a feature-based method for tracking a microrobot under an electron scanning microscope [52]. (c) An example of using an enhanced microscopic vision-based approach to support high-precision measurement on planar motions of a micro stage [46]. (d) A CNN-based method for depth and 3D orientation estimation for optical microrobots [53]. (e) An architecture of 6 DoFs pose estimation for optical microrobot using a ResNet-GPR hybrid model[51]. Panels (a),(e) reproduced from [51] with permission. Copyright 2020, American Chemical Society. Panel (b) reproduced from [52] with permission. Copyright 2009, SAGE Journals. Panel (c) reproduced from [46] with permission. Copyright 2019, IEEE. Panel (d) reproduced from [53] with permission. Copyright 2019, IEEE.

5: Examples of microrobots actuated by laser beam including with and without optical trapping. (a) The design of the microrobots with the shape of rotors and the experimental verification of the indirect manipulation via OT with hydrodynamic manipulation [63]. (b) Ultralow force surface scanning probe developed for the characterization of sensitive samples [64]. (c) Microtools functionalized with fluorescent streptavidin [65]. (d) Microrobot equipped with a thermoplasmonic disk for mixing in microfluidic channels. [66]. (e) Microrobot with a hollow-body syringe-like structure are actuated via thermoplasmonic-induced convection [59]. Panel (a) reproduced from [63] with permission. Copyright 2019, Springer Nature. Panel (b) reproduced from [64] under the terms of the CC-BY license. Copyright 2012, The Authors, published by Optica Publishing Group. Panel (c) reproduced from [65] with permission. Copyright 2012, Elsevier. Panel (d) reproduced from [66] under the terms of the CC-BY license. Copyright 2018, The Authors, published by Optica Publishing Group. Panel (e) reproduced from [59] with permission. Copyright 2016, Nature Publishing Group.

6: Sculpting the shape of the microrobots for the implementation of 6D manipulation actuated by conventional OT. (a) Illustration of 6-DoF real-time control of optical robots based on high-bandwidth 3-D actuation via multi-traps [75]. (b) The sketch and SEM image of microrobots with fork-like clamps actuated by HOT for indirect cell manipulation [76]. (c) Optical manipulation of the mobile articulated microrobot (Image obtained from [12]). (d) Two microrobots are used to rotate a micro-object for observation in cooperative manner [14]. Panel (a) reproduced from [75] with permission. Copyright 2019, IEEE. Panel (b) reproduced from [76] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Multidisciplinary Digital Publishing Institute. Panel (c) reproduced from [12] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Wiley. Panel (d) reproduced from [14] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Wiley.

7: Examples of using optical microrobots for various applications. (a) Cell manipulation using microrobot with spherical handles for optical trapping [96]. (b) Multiple microrobots are actuated by HOT for translational and rotational manipulation of filamentous cells in cooperative manner [97]. (c) A microrobot with tapered cylindrical shape, fabricated by TPP, has been designed as a passive force clamp [67]. (d) Microrobots for cell indentation experiments [98]. (e) Nut-like microrobot, which can be manipulated by optical traps for micro-assembly with a fixed screw [99]. (f) Assembly of two microparts with dovetails [100]. Panel (a) reproduced from [96] under the terms of the CC-BY license. Copyright 2019, The Authors, published by Multidisciplinary Digital Publishing Institute. Panel (b) reproduced from [97] under the terms of the CC-BY license. Copyright 2019, The Authors, published by Optica Publishing Group. Panel (c) reproduced from [67] with permission.

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8: Examples of using optical manipulation for *in vivo* applications. (a) Experimental setup for *in vivo* optical trapping of RBCs using OT [138]. (b) Schematic illustration, simulation and experiment results of actuation and tracking of microrobots with rocket-like shape in the blood. Image obtained from [36]. Panel (a) reproduced from [138] under the terms of the CC-BY license. Copyright 2013, Nature Publishing Group. Panel (b) reproduced from [139] under the terms of the CC-BY license. Copyright 2020, The Authors, published by Nature Publishing Group.

Progress and potential

Over the past few decades, technological advances in micro-robotics have demonstrated their potential in biomedical engineering. Optical manipulation is one of the most precise manipulation techniques, while optical microrobots bring together the advances in photonics and robotics to enable precise micro-scale trapping and manipulation by using light. Although optical micro-robotics techniques are promising tools, they are still in their infancy. For example, issues related to microfabrication of versatile optical microrobots with different materials and functions, robust perception and dexterous manipulation via the optical system, and biocompatibility represent major hurdles to overcome.

Supported by the latest advances in materials as well as 3D micro-fabrication microrobots with complex structures can be created with good reproducibility and yield, further expanding the use of OT for micro-object manipulation. In this review, recent progress of allied techniques, as well as the general trend of the development of optical microrobots are introduced, along with visual perception and manipulation of microrobots with OT. New capabilities and functionalities brought by materials for optical microrobots are also discussed in this review. Future research needs to address more versatile optical robotic systems, more sophisticated manipulation mechanisms to allow for self-assembly and collaborative control, as well as tangible biological applications. In these regards, issues related to new materials, actuation schemes, sensing mechanisms, and novel fabrication methods would underpin the future development of the field.

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