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A Micro-Tweezers System for Cell Isolation Task

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***Abstract*— This paper introduces a simple micromanipulation system with combination of passive-active release strategy for biomedical applications. To begin with a simple manipulation system, it is required to acquire the 3D position information for both end effectors and the interested particles. Successful pick - and - place task of small size micro-objects is invaluable but challenging. The domination of adhesion forces in micromanipulation especially during releasing task has been a common issue. A hybrid passive-active release strategy has been implemented in this system to mainly overcome adhesion forces. The proposed micro-tweezers system is a contact micromanipulation approach that has successfully manipulated silicon microbead (4 - 16 μm) with the aim to manipulate oocysts of parasite (4 - 5 μm).**

Keywords: Micro-manipulation, adhesion forces, micro-tweezers.

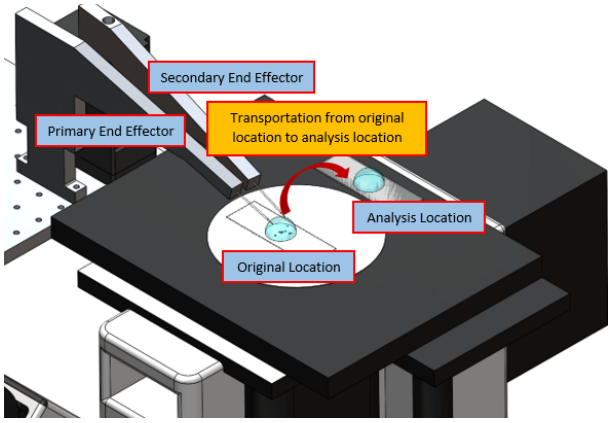

Fig. 1. CAD model of the manipulation system.

I. INTRODUCTION

Parasite-induced diarrhea kills thousands of children every year [1]. To understand which type of parasite is the real cause of this problem, parasite should be analyzed in single cell level. Recent studies indicated that, there might be some difference between parasites in the same culture [2], [3]. In this paper, a new parasite isolation mechanism is proposed to investigate each parasite comprehensively. In this way, it will be a first step to answer this important question: are all parasites are dangerous to human health or is it only certain ones?

Contact micromanipulation methods resort physical interaction to separate the micro-object from its culture and in literature, there are numerous examples of contact micromanipulation methods to handle a single cell. Most microgrippers are available in a variety of materials including polymers, alloys, ceramics, glass and metals with different functions, advantages and working mechanisms [4]. Electro-thermal microgripper works by applying an electric current through the structure that comprises of a narrow arm, a wide arm and a flexure [5]. There is the development of a microgripper that uses electrostatic actuator for manipulation. In order to move the gripper, the actuator contains a unique actuation structure of flexures to exert force through the gripper when a voltage is applied[6] - [9]. Although most microgrippers use direct contact while manipulating the particles [10] - [16], some microgrippers manipulate the particles through indirect contact such as microbubble. Manipulation of micro-objects using a microbubble assisted with an acoustic wave to attract or repel object from the microbubble has become popular because it does not damage the cells [17], [18].

In contact micromanipulation, there are two main challenges: the detection of the 3D position of the objects and dominance of adhesion forces. First, it is rather difficult to determine the position of the object in three-dimensional space from a 2D image that obtained from a conventional optical microscope camera. Second, the adhesion (surface) forces also become dominant over volumetric (gravity) force as the size of object is scaled down into micro-nano meter range [19]. As the adhesion forces increase, manipulating a smaller object is rather tricky through contact manipulation. Therefore, release strategy becomes essential criteria in contact micromanipulation, particularly for smaller micro objects (1 - 50 μm).

Release strategy comprises of two categories which are passive and active release methods. Typically, passive release methods deal with changing the features at end effectors, substrate or environment to minimise the adhesion forces. An increase in adhesion forces on the surface of the substrate to facilitate release is a good example but less reliable due to inconsistency of the relied forces for each release. Meanwhile, active release strategy is taking action during the releasing task [20]. Most micromanipulation systems nowadays incorporate active release strategy within the system, such as vibrating the tip of end effectors or plunging mechanism to overcome adhesion forces between the particles and the microgrippers, as if they are harmless yet sometimes can be complex [21] - [23].

In this study, a simple contact micromanipulation system which comprises of two end effectors has been proposed in order to achieve control over particles within the size of

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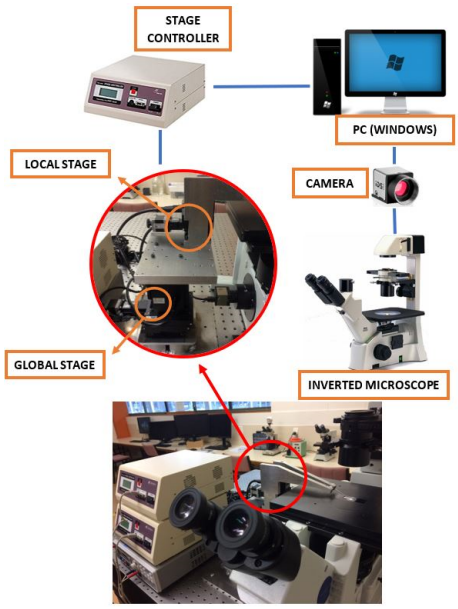

Fig. 2. System Configuration.

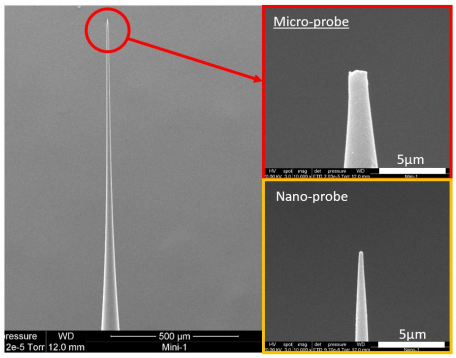

Fig. 3. Comparison between the thickness of micro-probe and nano-probe with $\sim$1.5μm tip diameter and $\sim$300nm tip diameter respectively.

TABLE I
SPECIFICATIONS OF MOTORISED STAGES.

	Local Stage	Global Stage
Travel (mm)	10(X,Y),5(Z)	50(X,Y),20(Z)
Resolution (μm)	1(X,Y),0.5(Z)	1(X,Y),0.1(Z)
Repeatibility (μm)	1(X,Y),5(Z)	2(X,Y),5(Z)
Max. Speed (mm/s)	10(X,Y),2(Z)	10(X,Y),1(Z)

5 μm to 10 μm (size range of oocysts (egg) of parasite). The proposed micro-tweezers system, as shown in Fig. 1, combines both nano and micro probes to overcome adhesion forces during releasing task. The system is also equipped with automated detection of 3D position for both target object and the tip of microgrippers which is in return leads to a reliable and consistent system. Developing a reliable and robust system to isolate micro-organism from cultural area would further assist microbiologists to explore and collect more detail genomic information of a single cell (oocyst of parasite) thus comprehensively apprehend the etiology of parasite-infected diseases.

This paper is structured as follows: Section II (materials and methods) introduces the system configuration for accomplishing a simple yet robust contact micromanipulation. Mitigation of adhesion forces and automated 3D position detection have also been described in this section. The experimental results about the simple contact micromanipulation are discussed in Section III. Eventually, conclusion is described in Section IV.

II. MATERIALS AND METHODS

A. SYSTEM CONFIGURATION

The experiment set-up consists of two motorised stages in which each of them possesses three micro-stepping actuators specifying the number of degree-of-freedom indirectly. The first stage is dedicated to global motion of the system such as moving both fingers. The second stage is responsible for local motion, i.e., the movement of a single finger for grasping and releasing the corresponding particles as shown in Fig. 2.

The two micro-stepping stages, which are local (TAMM40 and OSMS40 SigmaKoki) and global (OSMS80 and HPS80 Sigma-Koki) stages, offer submicron resolution with a large range as shown in Table I. These stages are integrated to an inverted microscope (Olympus IX71) with a microscope camera (The uEye, IDS). A basic User Interface was designed by in Visual Studio for image processing and automation algorithms. This interface enables the user to observe the working environment, identify the diameter of the objects and execute manual or autonomous pick-and-place operations. Any adjustment to enhance the performance can also be done from the user interface.

The end effectors are fabricated from Narishige PC-100 puller. The puller heats and pulls the end effector which is made from borosilicate glass capillary instantaneously. For nano tip diameter, a Sutter PC-2000 is used. The difference in tip diameter of these probes (Fig. 3) will assist the proposed hybrid passive-active releasing task.

B. INFLUENCE OF SIZE ON ADHESION FORCES

In microscale, adhesion (surface) forces are more significant than gravitational (volumetric) forces. So, grasping the particles can be performed easily as it is likely to stick [10]. There are three different forces within adhesion effects; van der Waals, electrostatic and capillary forces. The systems aim is to manipulate an oocysts of parasite in a liquid environment. In comparison of micro-object in liquid and dry mediums, the electrostatic and van der Waals are reduced due to the changes of the constant coefficients meanwhile the capillary force is eliminated as it immersed in liquid [24]. In this micromanipulation system, both end effectors will approach the targeted object followed by grasping. The contact between the object and the end-effector causes the targeted-object to adhere to one of the end effector which is mainly because of the van der Waals Forces (F_{vdw}) effect. The van der Waal forces, F_{vdw}, between a sphere and flat

surface objects is described by [25]:

$$F_{vdw} = \frac{AR}{6z^2} \tag{1}$$

Van der Waals forces are strongly affected by the Hamkar constant, A, of the materials other than distance between two objects. This Hamkar constant value in water is lower than the value in vacuum for some materials, thus reducing the value of the van der Waals forces within a very short range (typically ¡ 100 nm) compared to the size of the object (greater than 1 μm) [26]. R represents the spheres radius and z is the range distance between two objects. Meanwhile, F_{vdw} between two spheres is [25]:

$$F_{vdw} = \frac{AR}{12z^2} \tag{2}$$

As the end effectors appeared in conical shape and the targeted-object (microbead) is approximately to be spherical shape with smooth surface texture - although oocyts of parasite tends to have uneven surface texture - the van der Waals force between a conical and a spherical object (VDW_{c-s}) is described as follows [25]:

$$\frac{AR}{12z^2} < VDW_{c-s} < \frac{AR}{6z^2} \tag{3}$$

According to Eq. (3), contact area between two bodies (conical and spherical) strongly affects the amount of VDW forces [24]. Having different thickness of end effectors will thus control the micro-object adhering only at particular end effector entirely. In this way, we could apply a special releasing technique by pushing the micro-object off the end effector. A few bias-adhesion experiments were conducted over different sizes of probes. Hence, micro and nano probes with tip-diameter $\sim$1.5 μm and $\sim$300 nm respectively as shown in Fig. 3 worked well for bias-adhesion case compared to other probe sizes.

As the ultimate goal is to achieve manipulation of biological cells (oocysts of parasite), manipulation of silicon microspheres (SiO2MS, Cospheric) with similar size is experimented in a liquid environment. Summary of experiment results can be seen in Fig. 4 and in Table II. The experiment has been tested on 4 different size of objects: 4μm, 8μm, 12μm and 16μm under a 40x objective lens. In this section, six different combination of micro and nano probes have been presented. Two different releasing strategies are applied that are normal release strategy and special release strategy. Normal release strategy is the one where end effectors are moved away from each other using motorized stages. Special release strategy is the hybrid passive-active release approach. In this case, two different thickness end effectors are used and object is expected to adhere to thicker end effector naturally due to larger contact area. Then, by using tip of thinner end effector (minimum contact area), object is pushed away from the end effectors.

There are three different pairs of end effectors which include micro with micro, nano with nano and combination of micro and nano probes. In this manipulation system,

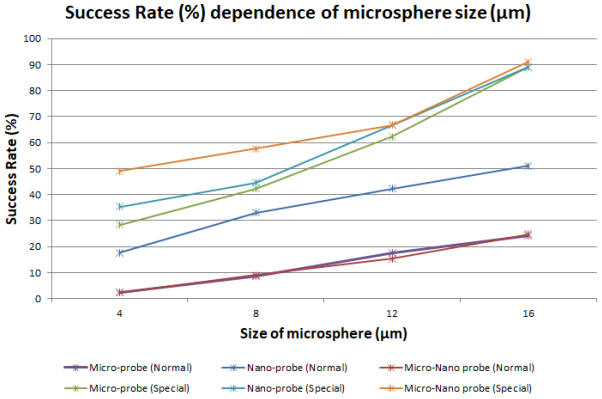

Fig. 4. A success rate (%) comparison for each micromanipulation technique.

TABLE II
MANIPULATION SUCCESS RATE (%) OF NANO-PROBE, MICRO-PROBE AND COMBINATION OF MICRO-NANO PROBE.

Micro-object	4μm	8μm	12μm	16μm
Normal Release Strategy				
Micro-probe	2.2%	8.7%	17.6%	24.3%
Nano-probe	17.6%	33.0%	42.3%	51.0%
Micro-Nano probe	2.2%	9.0%	15.3%	24.7%
Special Release Strategy				
Micro-probe	28.3%	42.3%	62.3%	89.0%
Nano-probe	35.3%	44.6%	66.7%	89.0%
Micro-Nano probe	49.0%	57.7%	66.7%	91.0%

a pair of end effector were used to grasp the targeted object, transport and release it at a specified location. The approach was assisted by the computer vision program used to detect the object and align the end-effectors. The object was delicately gripped to restrict the contact area between the end effectors and the microsphere in order to reduce the effective adhesion forces.

The experiments were continued by using a special release strategy that enables the left/right end effector to push the micro-object off from the right/left end effector, depending on which end effector the microsphere adheres to. A strong grasping has been applied in order to achieve contact area at its maximum [25]. Apart from merely transporting the micro-object in the X and Y directions, these experiments also consider lifting up the targeted object so that detaching the object from the effector can be easily detected as it tends to get blurred as soon as it touches the substrate. The experiments with different pair of end effectors were repeated 3 times with 15 trials for each microsphere in order to identify and analyse the variability and consistency of the outcomes.

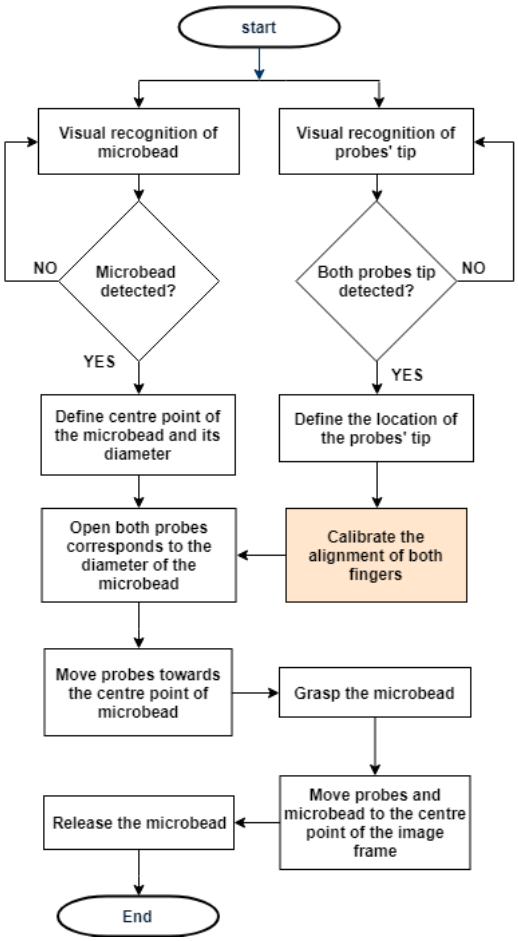

Fig. 5. Flowchart of the automated micromanipulation system.

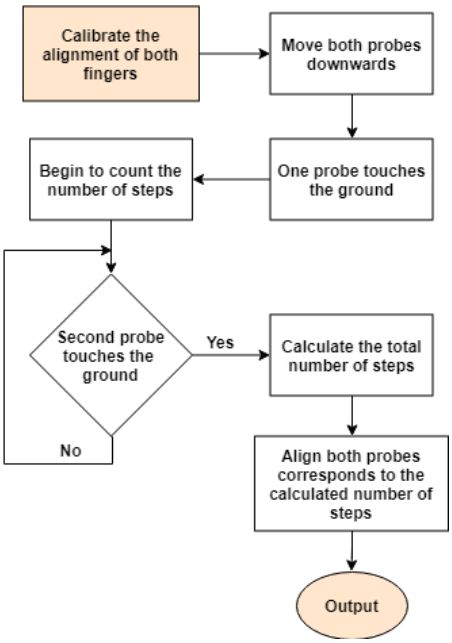

Fig. 6. Flowchart of the automated aligning for both end effectors.

C. ALGORITHMS FOR AUTOMATED MANIPULATION SYSTEM

In order to perform the micromanipulation task successfully, the location of the end effectors as well as the object should be defined beforehand. Measuring the position of the object in the X and Y axes (plane) is straightforward by analyzing microscopic images, however defining the depth level (Z direction) of the objects is a challenge. The Z direction is essential to calibrate both end effectors so that they are at the same level. In addition, grasping the microsphere requires the end effectors to be positioned at the microsphere's center plane. Due to the spherical shape of the microsphere, the depth level of its centre point above the substrate will always be its radius.

a) End-Effectors Detection: The end effectors are brought down (z-direction) into focus from the water surface while focusing the targeted objects. However, this does not mean that the end effectors would be at the same level of the targeted microsphere. To resolve the issue, the end effectors are moved down further until a little forward movement is noticed at the tips of the probes. This means, the probe tip has touched the ground. Thus, precise depth adjustment can be realised by having the end effectors to move upwards, corresponds to the radius of the targeted microsphere.

The end effectors tip is highly sensitive to the light intensity. Underexposed or overexposed image lead to undetected end effectors tip. The procedure of acquiring the interested region is as follows.

- Binary thresholding applied for every captured image;
- Detecting contour through Canny contour algorithm;
- Detecting the end effectors tip (x_{tip}, y_{tip}) by finding the left most point of the end effectors contour;

b) Micro-sphere Detection: The location of the targeted microsphere is found through Hough Circle transformation to detect circular shapes in the image. The parameters of the detected circular shapes are adjustable to specify the interested circular objects within the image. The Hough algorithm returns the radius and coordinate in the image of the detected object.

Based on the information obtained especially the diameter of the targeted object can be converted into actual measurement through pixel/metric ratio. The value of the pixel/metric ratio varies, corresponds to the size of objective lens. The conversion of measurements from camera space into global space is unfortunately restricted by the focus of the microscope and the resolution of the camera (1024 x 768 pixels) to accomplish consistent outputs. The magnification could also affect the measurement results, wherein at higher magnifications lead to less significant result.

An automated manipulation system is feasible after extracting three positions which are the location of both end effectors as well as the targeted micro-object. According to Fig. 5, the control sequence starts from visual recognition of the image coordinates of the end effectors tip and the targeted micro-object. Automated pick and place system begins with placing both end effectors just above the substrate. The

effectors are then moved to the centre of the targeted micro-object, corresponds to its radius. The automated manipulation continues by moving both end effectors towards the targeted object followed by grasping, transporting and basic releasing tasks. Furthermore, aligning of two end effectors is explained in Fig. 6

III. RESULTS AND DISCUSSION

A. RESULTS

The micromanipulation that includes grasp, transport and release task was completed by only using manual method through user interface, connected to the stage controller. The release techniques that had been used were two; basic release and special release. Special release method involves with pushing the object off the (left) end effector through the other (right) end effector [25].

The results indicate that the pushing (special) release technique performed better than normal release technique. This is because of the very high adhesion forces between the microsphere and the end effectors which means additional forces are required to detach it. A fairly large amount of force at high speed is applied during the special releasing technique to prevent the objects getting attached over the contact area. Most of the manipulations using special release technique have been demonstrated to perform at least 3 times better than normal release technique. This is shown in Table II. As well as this, the narrower the end effectors, it has lower associated adhesion forces compared to the thicker end effectors. Nano-probes ($\sim$300nm tip diameter) have a higher success rate of releasing the microsphere than micro-probes ($\sim$1.5μm tip diameter). The success rates of each experiment are shown in Table II. Each experiment consisted of 15 trials, with four different sizes of microbeads (4, 8, 12 and 16 μm diameter) being used. However, manipulating the objects using nano-probes is difficult due to difficulty calibrating the depth of the end effectors with the centre of the microsphere. Besides, the flexibility of the nano-probes, due to small thickness, makes them bend easily when high grasping force is applied against the microsphere. This leads to more contact area between the nano-probes and the microsphere, which results in high adhesion forces in return.

Implementing identical thickness of the end effectors affects the consistency of where the microsphere is going to attach to. The objects are adhered to the left end effector approximately 54% and 61% of the time for nano-probes and micro-probes respectively. The bias towards the left end effector might be because of it remains stationary while the grasping is solely being controlled by the right end effector. Besides, the angle of the right end effector ($\sim$15 degrees from horizontal) could possibly lead to less contact area during grasping [25]. Therefore, a combination of micro-nano probes has enabled the microsphere to adhere to the left end effectors approximately 90% of the time as shown in Fig. 7. Experimental video is also attached.

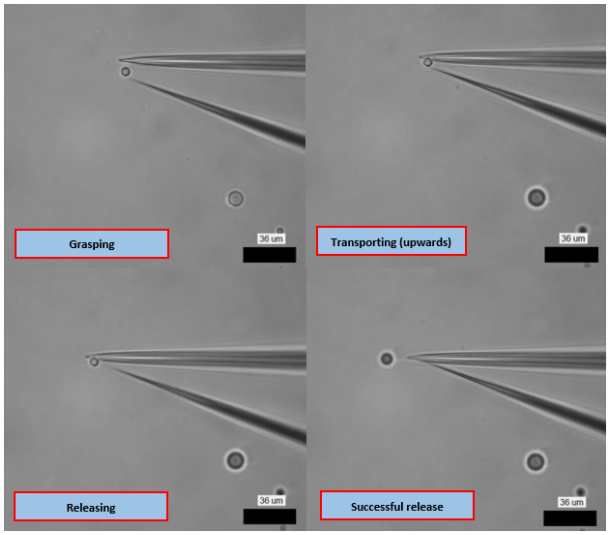

Fig. 7. Pick and place task for a 4 μm microbead using hybrid passive-active release strategy.

B. DISCUSSION

The experimental results show the proposed hybrid passive-active release strategy was superior than conventional release strategy for micromanipulation. There are a few basic pre-actions that lead to successful outcomes such as having the end effectors to align correctly in the z-plane. Not ideal grasping condition, such as grasping other than its centre, may lead to failure as the object tends to rotate and swing around as soon as it is moved in X and Y directions or brought a few steps above the substrate (Z direction).

This failure was significant on nano-probes due to smaller in size and less adhesion forces. The micro-probes were able to avoid the microsphere to swing and rotate around during releasing task. As a result, combination micro-nano probes was able to reduce the failure due to the object rotates around the left end effector [25]. Being able to control the location of the microsphere to adhere to was useful for automation.

IV. CONCLUSION

This study addresses the challenge of micro-manipulating smaller size microobjects (4 μm - 16 μm). A simple micromanipulation system with combination of micro-nano probes to facilitate release strategy (passive-active release strategy) was proposed with the aim to manipulate oocysts of parasite (4 μm) autonomously. Grasping and releasing were the challenging parts in this system. As the dealing size of object is small, the adhesion forces are dominant over the weight force. The implementation of nano-probe as the end effectors was successful however it requires more effort to obtain a perfect alignment especially during grasping. In contrast, micro-probe was observed to be more reliable during grasping but less reliable during releasing task due to large surface area and high adhesion forces. The system returns a better result when combining both micro and nano probes which is approximately 49% of success

rate for 4 μm size micro-object. The success rate could be improved by having a more consistent grasping scenario that requires a perfect alignment between the end effectors and the microobject.

In the future, we are planning to separate an inactive parasite from its colony. Thus, more experiments will be performed to optimise automation part especially autonomous alignment which involves acquiring information in z-direction.

REFERENCES

[1] Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., ... & Faruque, A. S. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet, 382(9888), 209-222.

[2] Grinberg, A., Biggs, P. J., Dukkipati, V. S. R., & George, T. T. (2013). Extensive intra-host genetic diversity uncovered in Cryptosporidium parvum using Next Generation Sequencing. Infection, Genetics and Evolution, 15, 18-24.

[3] Grinberg, A., & Widmer, G. (2016). Cryptosporidium within-host genetic diversity: systematic bibliographical search and narrative overview. International journal for parasitology, 46(8), 465-471.

[4] Sudhakar, R., Prathap, R. S., Sudarshan, K., & Vijay, S. (2016). Literature Review on Micro Grippers. Internation Journal of Emerging Trends in Engineering and Development, 6(6), 59-73.

[5] Pahwa, T., Gupta, S., Bansal, V., Prasad, B., & Kumar, D. (2012). Analysis & design optimization of laterally driven poly-silicon electro-thermal micro-gripper for micro-objects manipulation. Paper presented at the COMSOL Conf. Bangalore.

[6] Boudaoud, M., Haddab, Y., & Le Gorrec, Y. (2013). Modeling and optimal force control of a nonlinear electrostatic microgripper. IEEE/ASME Transactions on mechatronics, 18(3), 1130-1139.

[7] Xu, Q. (2015). Design, fabrication, and testing of an MEMS micro-gripper with dual-axis force sensor. IEEE Sensors Journal, 15(10), 6017-6026.

[8] Chang, H., & Hao, Y. (2017). A Rotary Microgripper. Micro Electro Mechanical Systems. Micro/Nano Technologies, 2, 2-18. doi:https://doi.org/10.1007/978-981-10-2798-7_27-1

[9] Chen, T., Sun, L., Chen, L., Rong, W., & Li, X. (2010). A hybrid-type electrostatically driven microgripper with an integrated vacuum tool. Sensors and Actuators A: Physical, 158(2), 320-327.

[10] E. Avci, T. Hattori, K. Kamiyama, M. Kojima, M. Horade, Y. Mae and T. Arai, "Piezo-actuated parallel mechanism for biological cell release at high speed," Biomedical Microdevices, vol. 17, no. 5, p. 98, 2015.

[11] Li, J., & Bi, Z. (2017). Micromanipulation Tools. In Advanced Mechatronics and MEMS Devices II (pp. 547-561): Springer.

[12] Liu, J., Shi, C., Wen, J., Pyne, D., Liu, H., Ru, C., . . . Sun, Y. (2015). Automated vitrification of embryos: A robotics approach. IEEE Robotics & Automation Magazine, 22(2), 33-40.

[13] Ciubotariu, D. A., Ivan, I. A., Clvy, C., & Lutz, P. (2016). Piezoelectric 3D actuator for micro-manipulation based on [011]-poled PMN-PT single crystal. Sensors and Actuators A: Physical, 252, 242-252.

[14] Liang, C., Wang, F., Shi, B., Huo, Z., Zhou, K., Tian, Y., & Zhang, D. (2018). Design and control of a novel asymmetrical piezoelectric actuated microgripper for micromanipulation. Sensors and Actuators A: Physical, 269, 227-237.

[15] Xu, Q., Wan, Y., Hu, T. S., Liu, T. X., Tao, D., Niewiarowski, P. H., . . . Yang, Y. (2015). Robust self-cleaning and micromanipulation capabilities of gecko spatulae and their bio-mimics. Nature communications, 6, 8949.

[16] Zhang, Y., Yu, Y., Zhang, Z., & Zhang, X. (2017, July). Structure and Design of Microgrippers: A Survey. In Cybernetics, Robotics and Control (CRC), 2017 2nd International Conference on (pp. 139-143). IEEE.

[17] Park, I. S., Shin, J. H., Lee, Y. R., & Chung, S. K. (2016). On-chip micromanipulation using a magnetically driven micromanipulator with an acoustically oscillating bubble. Sensors and Actuators A: Physical, 248, 214-222.

[18] Shin, J. H., Seo, J., Hong, J., & Chung, S. K. (2017). Hybrid optothermal and acoustic manipulations of microbubbles for precise and on-demand handling of micro-objects. Sensors and Actuators B: Chemical, 246, 415-420.

[19] Fearing, R. (1995). Survey of sticking effects for micro parts handling. Proceedings 1995 IEEE/RSJ International Conference on Intelligent Robots and Systems. Human Robot Interaction and Cooperative Robots.

[20] Kim, E., Kojima, M., Kamiyama, K., Horade, M., Mae, Y., & Arai, T. (2018). High-Speed Active Release End-Effector Motions for Precise Positioning of Adhered Micro-Objects. World Journal of Engineering and Technology,06(01), 81-103. doi:10.4236/wjet.2018.61005.

[21] Zhang, Y., Chen, B. K., Liu, X., & Sun, Y. (2010). Autonomous robotic pick-and-place of microobjects. IEEE Transactions on Robotics, 26(1), 200-207.

[22] Chen, T., Wang, Y., Yang, Z., Liu, H., Liu, J., & Sun, L. (2017). A PZT Actuated Triple-Finger Gripper for Multi-Target Micromanipulation. Micromachines, 8(2), 33.

[23] Demaghsi, H., Mirzajani, H., & Ghavifekr, H. B. (2014). A novel electrostatic based microgripper (cellgripper) integrated with contact sensor and equipped with vibrating system to release particles actively. Microsystem technologies, 20(12), 2191-2202.

[24] K. F. Bhringer, R. S. Fearing, K. Y. Goldberg, Handbook of Industrial Robotics, 2nd ed., John Wiley & Sons, New York, Ch. 55, pp. 10451066, 2007.

[25] Crimp, D. (2017). Development of A Micromanipulation Platform for Single-Cell Separation (Honour years thesis). New Zealand, Massey University.

[26] Gauthier, M., Rgnier, S., Rougeot, P., & Chaillet, N. (2006). Analysis of forces for micromanipulations in dry and liquid media. Journal of Micromechatronics, 3(3-4), 389-413.

[27] P. Lambert, and S. Rgnier, Surface and contact forces models within the framework of microassembly, Journal of Micromechatronics, vol. 3, no. 2, pp. 123-157, 2006.