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Dynamic Releasing of Biological Cells at High Speed Using Parallel Mechanism to Control Adhesion Forces

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Abstract— **In this paper, a dynamic releasing method for high-speed biological cell manipulation is proposed. A compact parallel mechanism, used for grasping and releasing microobjects, was utilized for generating controllable vibration to overcome the strong adhesion forces between the end effector and the manipulated object. To reach the required acceleration of the end effector, which is necessary for the detachment of the target object, vibration in the end effector is generated by applying sinusoidal voltage to the PZT actuator of the parallel mechanism. For the necessary acceleration, we focus on the frequency of the vibration, while keeping the amplitude of the PZT actuator vibration small (14 nm) to achieve precise positioning. Releasing of microbeads and biological cells is conducted and results are compared for the first time. The effect of the air and liquid environments are also investigated. Successful releasing (97.5%) of biological cells proves that the proposed active releasing method is an appropriate solution for the adhered biological cells during the releasing task.**

I. INTRODUCTION

The extension of robotic pick-and-place techniques from macro to microscale is a complex task. The manipulation at microscopic scale adds convenience, but brings on new challenges. One of the strong points of the micromanipulation is the ease in handling a microobject (particularly when the size is smaller than 50 μm), even with one end effector, due to adhesion forces [1].

On the other hand, depth of view, inverse proportionality of resolution and field of view for optical microscope, limited space for end effectors, miniaturization of the entire robotic structure could be accepted as difficulties of micro-scale manipulation. However, among the challenges, a particularly difficult issue is the stickiness between the microobject and the end effector during the releasing task, resulting from the dominance of the surface forces [2]. Scaling down the forces from macro to micro leads surface forces (adhesion forces) consisting of the van der Waals, capillary and electrostatic forces, to dominate volumetric forces, e.g., gravity [3]. Adhesion forces being attractive, the handled microobjects tend to stick to the end effector, making releasing very hard if not impossible. It does not matter whether the system uses one [4], two [5] or multiple end effectors [6] to achieve micromanipulation, adhesion forces stand as a challenge to be overcome for the releasing task.

In our recent work, a new compact parallel mechanism to decrease the undesired vibration of end effectors during high-speed motions was proposed [7]. We also introduced the manipulation of microobjects at high speed using a two-fingered microhand to achieve a 3D cellular structure [8]. Although grasping and transportation microbeads (40-60 μm) actions were conducted with a high success rate, the results of the releasing task were not satisfactory (70%). The success rate of the release would be lower in case of manipulating smaller size objects, as the effect of adhesion forces is inversely proportional to the size of the microobjects. In our current work, to achieve a complete pick-and-place task with a high success rate, a fast and robust technique to detach the target object from the end effector is studied.

For the detachment of microobjects from the end effector, various methods, which can be categorized as passive and active release, have been proposed. Passive release depends on changing the features of the end effector, substrate or environment. Coating the substrate with gold [9], coating the end effector chemically [10], surface roughening of microgrippers [11], controlling the pH value of the liquid environment [12] are a few examples of passive release. However, this method mostly depends on surface properties. In addition, changing the features of the manipulation tools and environment would not be appropriate for biological cell handling in terms of bio-compatibility.

On the other hand, applying a voltage between the end effector and the substrate to produce an electric field [13], using a vacuum tool to create a pressure difference for picking up and releasing [14] are two different cases of active release. Among the various techniques of active release, the detachment of the target object from the end effector utilizing the motion of the end effector has drawn much attention due to its high success rate [2], [15], [16].

Chen et al. [2] achieved the release of 7.5-10.9 μm size borosilicate glass spheres in air by integrating a plunging mechanism to the gripper to impact the microobject so that it gains sufficient momentum to overcome adhesion forces. The success rate of the releasing task was 100%. Haliyo et al. [15] accomplished releasing 40 μm radius glass spheres in air by taking advantage of the inertial effects of both the end-effector and the manipulated object to overbalance adhesion forces. The high acceleration required for the release is produced by PZT ceramics. Recently, 20-100 μm

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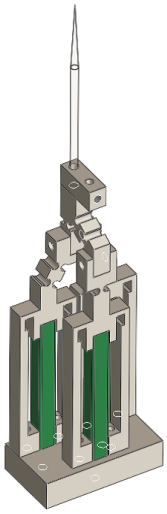

Fig. 1: Compact parallel mechanism, not only for grasping and releasing but also for mechanical vibration.

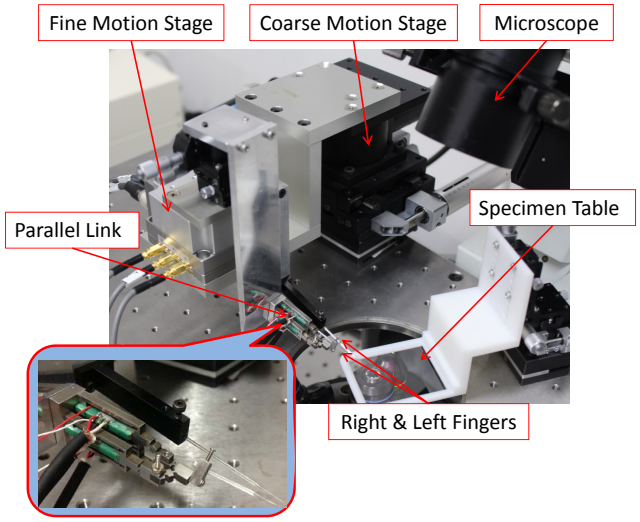

Fig. 2: System configuration.

polystyrene spheres were released in air with a 100% success rate based on the adhesion control with compound vibration [16]. A piezoelectric ceramic module was attached to the microgripper to produce controllable vibration in the vertical axis. The aforementioned three studies utilize the motion of the end effector to detach manipulated objects. All three studies require additional actuation mechanisms to produce the necessary speed and acceleration. In addition, all systems were tested with microbeads and manipulation environment was air in all cases.

To the best of our knowledge, there is no active vibration method for the robust releasing of biological cells. In this paper, we present a compact parallel link mechanism, i.e., a microhand which has three PZT actuators to provide 3-DOF to the end effector for grasping and releasing microobjects (Fig. 1). With this parallel mechanism, creating vibration for the active release is feasible. Thus, no additional actuation mechanisms for the releasing task are required. To use the inertial effects of both the end effector and the manipulated object for overcoming the adhesion forces, mechanical vibration for the end effector is generated by the PZT actuators of the parallel mechanism. This microhand has the ability to release biological cells by utilizing the rapid acceleration of the end effector. As the target object is a living cell, the manipulation environment is a liquid. In [16], the optimum amplitude of the vibration for detaching the target object was investigated, whereas we focus on the frequency of the vibration, while keeping the amplitude of the vibration small (14 nm) to achieve precise positioning.

II. Materials and Methods

A. Experimental Set-Up

In this section, we introduce the experimental setup for high-speed micromanipulation. Fig. 2 shows the configuration of the micromanipulation system. A coarse motion stage (Sigma-Koki, TSD-805S) and a fine motion stage (Sigma-Koki, SFS-H60XYZ) are used for the global motion, that is the movement of both end effectors. With the global motion, the transportation of the target objects and the positioning of the microhand can be achieved. A combination of these two stages helps to realize the large workspace and precise motion necessary for the manipulation task, as illustrated in Table I.

TABLE I: Specifications of motorized stages.

	Fine Stage	Coarse Stage
Travel(mm)	0.1(X,Y,Z)	25(X,Y),10(Z)
Resolution(μm)	0.01	1
Accuracy (μm)	0.1	17
Repeatability (μm)	0.15	2
Max. Speed (mm/s)	2.3	0.1

The parallel link mechanism is for the local motion, in which grasping and releasing different-size target objects can be achieved. In this study, the same parallel mechanism is used for producing mechanical vibrations at the end effector, too. This compact mechanism is the modified version of the 3-Prismatic-Revolute-Revolute (3-PRR) parallel mechanism [7]. As the parallel mechanism is very compact with high stiffness, there is no significant vibration during high-speed motion. Furthermore, due to high stiffness, it is possible to control frequency and amplitude of generated vibration robustly. The mechanism includes 3 piezo actuators (NEC TOKIN, AE0203D16) as prismatic joints, which can be extended up to 10.7 μm. The workspace of the right end effector, connected to the parallel link, is 264x66x18 μm^3 with sub-micrometer precision. The parallel mechanism has the following merits: high speed, high accuracy and high rigidity, in addition to its simple and compact configuration. The mechanism possesses a 3-DOF end effector (two rotational and one translational motions).

The rough and the fine stages are controlled by a Linux PC

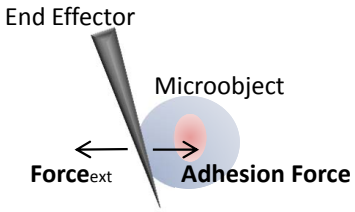

Fig. 3: Adhesion forces between the microobject and the end effector.

(Dell, XPS600, Pentium 4 3.80 GHz) through commercially available stage controllers (Sigma-Koki: Omec-4BG, Fine-503). The parallel link mechanism is controlled by the same PC through a D/A board (Contec DA16-16(LPCI)L) and a drive amplifier (MATSUSADA, HJPZ-0.15Px3). The displacements are measured with a strain gauge attached to the piezoelectric devices, and sent to the PC through a strain amplifier (Kyowa MCD-16A) and an A/D board (Contec AD16-16(PCI)EV) for PID control, in order to compensate the hysteresis effect of the piezo actuator.

The two end effectors of the microhand and the target object are observed under an IX81 motorized inverted optical microscope using an Olympus LUCPlanFLN 20x/0.45na Ph1 objective lens. The images are captured by a high-speed camera (Photron FASTCAM MC2) and displayed on a Windows PC (Intel Core i7 CPU, 2.93GHz with 4 GB RAM) monitor. The piezo actuator, attached to the objective lens, controls the motion of the objective lens to keep the target in focus. The end effectors, –the right and left fingers– of this micromanipulator consist of two glass needles which have a 23 mm length, 1 mm diameter and sharpened ends with less than 1 μm curvature.

B. Effect of Adhesion Forces on Micromanipulation

The dynamics of microscopic objects are different from macroscale objects. As length(l) of objects decrease from macro to microscale, surface forces (l^2) begin to dominate body forces (l^3). Because gravitational forces are proportional to the object volume whereas adhesion forces are proportional to the object surface, adhesion becomes dominant compared to gravitational force in the microscale [3]. The adhesion forces include van der Waals forces, electrostatic forces, and surface tension forces.

As gravity can be neglected in this scale, only the adhesion force (W) needs to be overcome by the acceleration(a) of the end effector [17].

$$F_{ext} = ma > 2R_b\pi W. \quad (1)$$

In (1), F_{ext} stands for the force required to detach the end effector from the microobject. R_b represents the radius of the microobject. The effect of the adhesion force to the end effector and the manipulated object can be seen in Fig. 3.

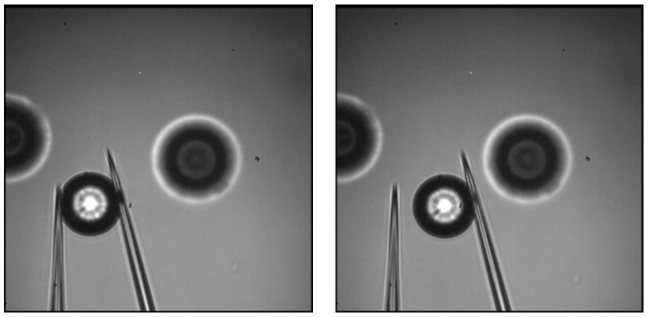

Fig. 4: Release attempt of 55 μm microsphere with two fingered microhand without any special method, a) before release attempt, b) after release attempt.

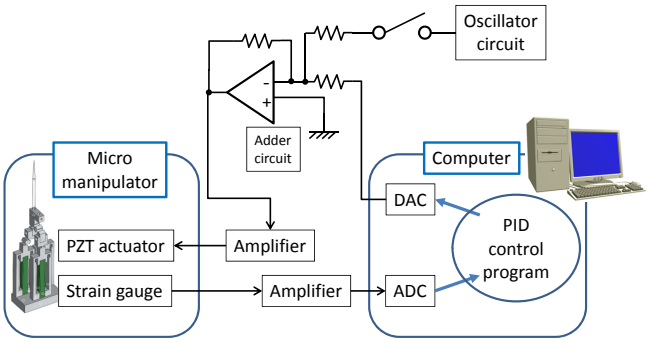

Fig. 5: Control of parallel mechanism for grasping, releasing and active vibration.

In Fig. 4, release attempt for the 55 μm microsphere with two fingered microhand without any special method is shown. As it can be seen, after opening the end effectors, microbeads was adhered to the right finger. As the effect of adhesion forces is inversely proportional to the size of the microobjects, releasing of smaller size microobjects is more difficult in the absence of a particular method.

C. Controllable Vibration for the Active Release

To release the manipulated object attached to the end effector, a controllable vibration in the finger is produced by the PZT actuator of the parallel link mechanism. If the end effector can reach the required acceleration, overcoming the adhesion forces (detachment) is possible due to the inertial effects of both the end effector and the manipulated object.

Fig. 5 illustrates the control scheme of the parallel link mechanism, i.e., the right end effector. To move the end effector to the desired position, a necessary voltage is applied from the computer via a Digital-to-Analog Card (DAC) and an amplifier (Piezo Driver). Through strain gauge, the extension of each PZT actuator is sent to the same computer via another amplifier and an Analog-to-Digital Card (ADC). With this feedback mechanism, PID control is applied, in

order to achieve a robust positioning.

On the other hand, the same parallel link mechanism is used to generate vibration through the PZT actuators of the parallel mechanism. For this, a custom-made oscillator circuit is integrated to control the scheme as seen in Fig. 5. The On-Off switch of the oscillator circuit is controlled by the computer. The type of signal sent by oscillator circuit is sinusoidal.

From the peak acceleration formula [16], if the frequency (f) and amplitude (A) are known, the required acceleration (a) can be calculated.

$$a = 4\pi^2 f^2 A. \tag{2}$$

In [16], the optimum amplitude was investigated by fixing the frequency. However, in our study, to release the manipulated object with a small deflection of the end effector, we set the amplitude to a small but sufficient to release value [15]. To calculate the amplitude of the end effector vibration, the following steps are conducted.

$$E_c = (E_m V_c)/V_m. \tag{3}$$

In (3), E_m represents the maximum extension of the PZT actuator –10.7 μm– in the case of applying the maximum voltage (V_m) of 150 V. E_c stands for current extension, while V_c stands for current voltage, i.e., 200 mV. Thus the current extension of the PZT actuator is 14 nm.

To calculate the current amplitude of the vibration, the following equation is utilized.

$$D_c = (D_m E_c)/E_m. \tag{4}$$

In (4), D_c represents the current deflection of the end effector from the original position, where D_m stands for the maximum deflection (141.6 μm) of the end effector, which can be obtained from the workspace of the parallel mechanism. Consequently, the current deflection is calculated as 0.18 μm. However, from the image feedback, the deflection amount is calculated as 1 μm. Being close to the resonance frequency of the end effector could be a possible reason, which will be considered in the future work.

As the amplitude of the vibration is obtained, the optimum frequency for successful releasing can be investigated. Thus, we can calculate the required minimum acceleration for the releasing task in the next chapter.

III. Experimental Results and Discussion

Experiments with the various size microbeads (10 μm, 20 μm, 55 μm, 97 μm) and 3T3 mouse cell (13 μm) have been conducted. For the 10 μm - 20 μm microbeads and 13 μm cell, 40x magnification is used which makes visible space as 128 μm x 128 μm. On the other hand, for the 55 μm and 97 μm microbeads, 10x objective lens is used and that provides visual space of 512 μm x 512 μm. Releasing experiments have been done just above (height was 1-2 μm) the substrate to not be affected by air flow and fluid flow. Room temperature was set to 24 °C.

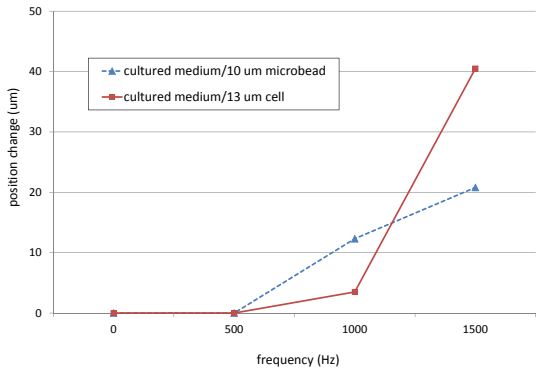

Fig. 6: Effect of frequency to the release of microobjects.

Current work is a part of study about high-speed micromanipulation [8]. As in the previous work [8], high-speed grasping and transportation of various size microobjects was achieved, on this paper, we focus on the fast releasing strategy. Thus releasing part of the micromanipulation task is discussed here.

In the following experiments, optimum frequency for various size microbeads and 3T3 mouse cells have been investigated. Releasing in different environments such as air, water and culture medium has been conducted to understand the effect of the environment to the required amount of frequency and success rate of the task. At the end of experiments, it was possible to compare active release results for the microbeads and the biological cell.

A. Active Releasing at High Speed

First, different amount of frequencies were applied to the adhered microbeads and mouse cells to understand the effect of the changes in frequency and thus in acceleration.

In Fig. 6, two different objects (10 μm size microbead and 13 μm size mouse cell) releasing in culture medium is illustrated. When the frequency of the PZT actuator was increased from 0 Hz to 500 Hz, manipulated objects were still adhered to the end effector. Then the frequency was increased from 0 Hz to 1 kHz and the microbead was detached with 12 μm positional change, where 3T3 cell is detached with 3.5 μm positional change. Finally, when the frequency is increased from 0 Hz to 1.5 kHz, this time, the microbead was released with 20 μm positional change and the cell was released with 40 μm positional change.

From the Fig. 6, significance of an appropriate amount of frequency can be realized. When the applied frequency is less than the necessary amount, releasing is not possible. When the applied amount is more than the required value, the positional change of the microbeads and biological cell is enormous due to the inertia of both the end effector and manipulated object. Therefore, necessary frequency for successful release is investigated for different size microbeads and 3T3 mouse cell.

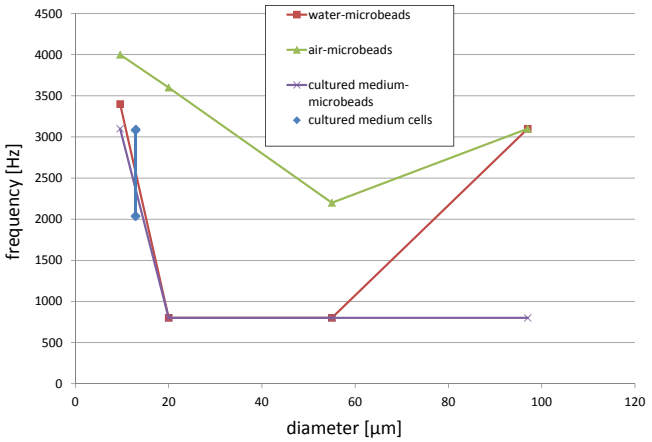

Fig. 7: relationship of required frequency and size of the manipulated objects in different environments.

TABLE II: required acceleration for successful release in different environments.

	Microbeads diameter(μm)	9.6	20	55	97
Air/ microbeads	Release frequency[Hz]	4000	3600	2200	3100
	Release acceleration value(m/s^2)	631	511.1	190.9	379
Water/ microbeads	Release frequency[Hz]	3400	750	750	3100
	Release acceleration value (m/s^2)	455.9	22.2	22.2	379
Cultured medium/ microbeads	Release frequency[Hz]	3100	750	750	750
	Release acceleration value(m/s^2)	379	22.2	22.2	22.2
Cultured medium/ cells	Release frequency[Hz]	2000-3100			
	Release acceleration value(m/s^2)	157.8-379			

For measuring the required frequency in different situations, four different size microbeads were released by generating vibration at the end effector in air, water and culture medium. For the mouse cell case, release task was conducted in culture medium as that is the only appropriate environment for biological cells.

In Fig. 7, in general, when the size of the microbeads is bigger, the required frequency for release is smaller. Therefore, size of the adhered object and the required frequency to release are inversely proportional. Another outcome of the experiment was necessity of the bigger frequency for releasing the microbeads in air compare to pure water and culture medium environments. Latter two environments are required similar frequencies.

For releasing the 3T3 mouse cell, the necessary frequency was about 2-3 kHz. It is not possible to apply exactly same amount of frequency to release biological cells for different trials as can be seen in Fig. 7. A possible reason for this uncertainty is as follows. During the grasping task, the contact area between the end effector and microbeads is not changing every time as microbeads have hard structure. On the other hand, as cells are very soft, during the grasping task, it is easy to squeeze the cell. According to squeezing amount, contact area between the end effector and manipulated cell is altering. Thus the amount of the adhesion force is changing too. Consequently, it is not feasible to apply a fixed amount of frequency to achieve high success rate of biological cell releasing. Adding a force sensor, which was manufactured and experimented in our previous work [18], would be helpful to measure adhesion force, thus to estimate the required amount of frequency.

In Table II, the required frequencies to release multi-sized microbeads and the mouse cell are shown. By using (2), the required accelerations according to found frequencies are calculated as seen in Table II. Required duration for the successful release is realized as 10 ms. If vibration applied for 10 ms, releasing the manipulated objects was always achieved. After finding the necessary frequency for each case, success rate of the strategy was 97.5 % out of 360 trial.

Dynamic releasing of a mouse cell is shown in Fig. 8. The successful release of the cell has been achieved consecutively two times by applying the vibration to the end effector. After opening the end effectors, the adhered cell to the right end effector is shown In Fig. 8 a). In Fig. 8 b), the released cell just after applying the vibration is illustrated. In Fig. 8 c), the right end effector is moving away to show release was successful. Then the right end effector is coming closer to the cell again and touch it. Thus the cell is adhered to the right finger again. In Fig. 8 d), the end effector is moving to the right direction with adhered cell to show stickiness. In Fig. 8 e), vibration is applied again and cell is released for the second time. In Fig. 8 f), the right end effector is moving away from the released mouse cell.

B. Discussion

By generating vibration in the end effector with the PZT actuator and adjusting the vibration frequency with respect to the size of the object, kind of the object and environment, successful release is confirmed through experiments. Applying vibration to release biological cell was done for the first time. And to produce vibration, no additional actuation mechanism was needed unlike the related works as the actuators to move the end effector have ability to produce vibration.

During the experiments, effect of the environment was investigated. When the end effector touches to the target object, probability of sticking depends on the environment (in culture medium $>$in air $>$in water). However, after sticking, the required frequency in each environment is in different order (in air $>$in water $>$in culture medium).

For precise placing, adhesion force information is important as it is not same all the time. Therefore, adding a force sensor to the system is our next work.

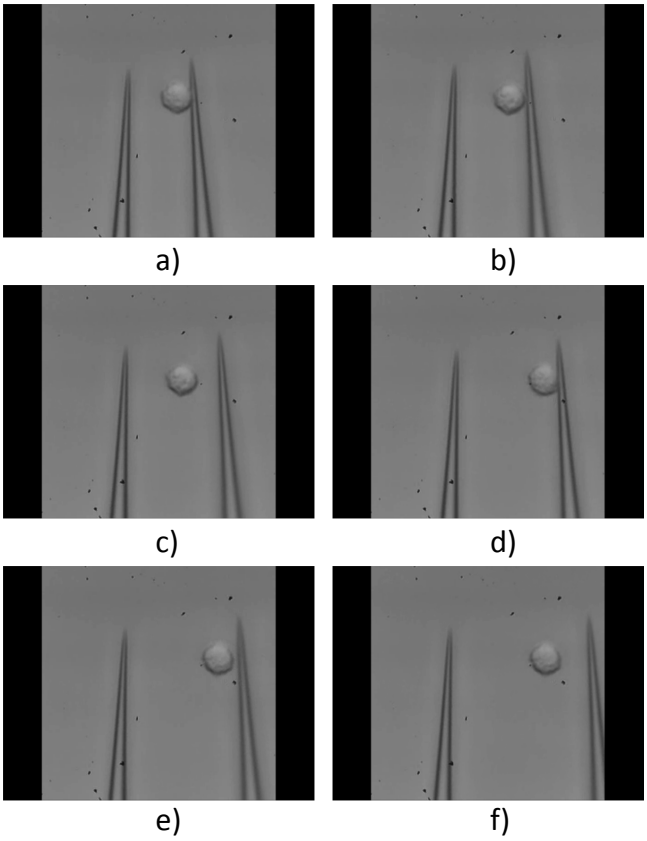

Fig. 8: dynamic releasing of 13 μm 3T3 mouse cell.

In current study, availability of the vibration as a release method is confirmed for multi-sized microbeads and biological cells. However as manipulated object would stick either of the end effectors, controlling both fingers with the parallel mechanism is feasible solution. In addition, controlling both end effectors with PZT actuators offers new abilities to the microhand for more complex manipulation tasks, e.g., rotation or turnover of the target objects.

IV. Conclusions

To release the microobjects, an active release method – controllable vibration– is proposed. No additional actuation mechanism is needed as the mechanism to control the end effector position includes 3 PZT actuators which can produce high frequency vibration. By fixing the amplitude of the vibration, the required frequencies under different conditions are investigated. Thus, the needed accelerations are calculated according to frequency results.

Releasing of the microbeads and the biological cells (first time) is conducted. The required frequencies for releasing in air, water and culture medium are analyzed. Comparison shows that the required frequency decreases with the increasing size of microbeads. In addition, for the similar size microbead and mouse cell, similar level of frequency is necessary. However for finding optimum frequency to release the cell, a force sensor can be integrated to the end effector to calculate exactly necessary frequency for each time.

After reaching the certain amount of frequency, 97.5% success rate for releasing is achieved. The accuracy of the placing will be studied in the next work.

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