Evaluating the Role of Force Feedback for Biomanipulation Tasks[*]

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ABSTRACT

Conventional cell manipulation techniques do not have the ability to provide force feedback to an operator. Poor control of cell injection force is one of the primary reasons for low success rates in cell injection and transgenesis in particular. Therefore, there exists a need to incorporate force feedback into a cell injection system. We have developed an automated cell injection system, which has the capability of measuring forces in the range of μN. We tested our system with 40 human subjects to evaluate the role of force feedback in cell injection task. Our experimental results indicate that the subjects were able to feel the cell injection force and confirmed our research hypothesis that the use of combined vision and force feedback leads to higher success rate in cell injection task compared to using vision feedback alone.

CR Categories: J.2 [Physical Science and Engineering]

Keywords: Cell injection, Force feedback, Transgenesis.

1. INTRODUCTION

Manipulating individual biological cell is a common process involved in intracytoplasmic sperm injection (ICSI), pro-nuclei DNA injection, gene therapy, and other biomedical areas. Potential application involves regional or target specific delivery of genetic material within a cell or an embryo. Researchers in the field of biology have manipulated individual cell using conventional manipulation techniques [1, 2]. However, manual manipulation requires long training and the success rate depends on the experience of the operator. Even for an experienced operator, the injection process results in low success rate and poor reproducibility, since the outcome of transgenesis task is not related to successful injection itself but rather the successful integration of the genetic material into the genome within the nucleus as a stable transfection. Efficiency of intracellular injection can be improved by providing the operator with an accurate haptic ("feel") and visual feedback from the cell. A haptic and visual feedback system can be used to manipulate an individual cell or an array of cells and standardize the outcome of cellular injection procedures. Transgenic techniques have been in use for 20 years for the creation of 'knockout mice'. These procedures are straightforward but technically challenging and the

transformation and survival rate are typically around 20% [3]. Typical transgenic organisms are created by introducing modified genetic material mechanically, one cell at a time. This method is preferred because it introduces the gene of interest along with the desired regulatory sequences without introducing other potentially confounding compounds. Alternative approaches for gene delivery include viral vectors, electroporation and liposomal carriers [4]. Current transgenic technology is labor intensive and has relatively low yield. Hence a need exists to develop an accurate and reliable cell injection system.

There has been limited work in the literature to automate the cell injection process using either vision feedback alone or combining it with force feedback. Piezo actuators were proposed for micromanipulators because of their high control bandwidth and smart structure design [5-7]. Active vision techniques have been investigated to develop an automated biomanipulation system [8-10]. Apart from mechanical manipulation, researchers have also explored various other methods. Optical trap is one of the promising methods to manipulate microscopic objects without physical contact. This technique may result in possible damage of the cell from absorption of high energy and subsequent heating after exposure to the trap [11]. The other manipulation techniques are magnetic, acoustic and electric field [12-14]. Even though there have been considerable efforts to automate manipulation of biological cells, vision has been the primary sensing modality. Few researchers proposed the concept of "bilateral control" in micromanipulation, which involves a master-slave set up [15, 16]. But the experiments were performed on rigid objects and the forces measured were in the range of Newtons, where as for biological cells the puncturing forces are of the order of μN-mN [17, 18]. A nanomanipulation system has been developed to provide force feedback from biological samples and carbon nanotubes [19]. In this set up the user does not feel the actual forces from the sample, but feels a surface representation that is simultaneously reconstructed during the scan. Zappe et al [20] developed an automated embryo injection system, which demonstrated 96% success in fruit fly embryo injections. However, there were no human factors studies to evaluate the benefit of force feedback in the literature.

We hypothesize that combining force feedback with vision can lead to better outcomes of cell injection tasks. Towards that goal, we have developed a force feedback interface to provide real time force feedback to the user and tested the system with 40 human subjects by doing separate cell injection experiments with only vision (V) feedback and vision + force (V+F) feedback. Since zebrafish is an excellent model for vertebrate studies, we performed experiments on zebrafish egg cells with the eventual goal of developing an automated transgenic manipulation. The paper consists of four sections. In section 2, we present the materials and methods used in our work. In section 3, we present the results from our experimental work with human subjects. Finally, in section 4, we make some concluding remarks and the direction for future work.

2. MATERIALS AND METHODS

The biomanipulation system (see Fig. 1(a)) consists of a nanomanipulator (Model: MP-285, manufactured by Sutter, Inc.) forming the cell injection unit and a micromanipulator (Model: TransferMan NK2, manufactured by Eppendorf, Inc.) forming the cell holding unit. The nanomanipulator as well as the micromanipulator has 3-DOF each and the inverted microscope (Model: IX81, manufactured by Olympus, Inc.) has a 2-DOF stage for positioning the sample. The travel range for MP-285 nanomanipulator (computer controlled) is 25mm along all three axes (X, Y & Z) with lowest resolution of 0.02 μm/step and highest resolution of 40 nm/step. The travel range for TransferMan NK2 micromanipulator (joystick controlled) is 20mm in all three axes with a resolution of 40nm per step. We used PVDF (polyvinylidene fluoride) film to develop the force sensor for measuring the cell injection forces. PVDF film is ideal for our application because of excellent sensitivity, high compliance and high signal to noise ratio [21, 22]. As shown in Fig. 1 (b), the glass micropipette is integrated onto the PVDF film (Thickness: 28μm, Model: LDT1-028K of MSI, Inc.) with the help of a connector. This set up allows easy removal and replacement of the micropipette if its tip (5μm ID) gets damaged during micromanipulation. The injecting pipette is connected to a pneumatic PicoPump (Model: PV830, manufactured by WPI, Inc.) for the purpose of injecting blue dye into the cell (refer to Fig. 2). A manual piston pump (Model: CellTram Air, manufactured by Eppendorf, Inc.) is used to apply suction for reliable holding of suspended zebrafish egg cells. The cell injection system is integrated with vision and haptic interface (see Fig. 3). The charge from the PVDF film is amplified by the charge amplifier (Model: 5010B, manufactured by Kistler).

A theoretical model for the PVDF film is developed and compared with the experimental calibration [23]. The PVDF film is calibrated with the load cell (Model: GSO-10 of Transducer technology Inc., maximum measurement range: 98.1mN and accuracy of 50 μN). A linear relationship is established between the applied force and the corresponding integral voltage output from the charge amplifier as shown in Fig. 4. The mathematical relationship is given by:

$$F = 0.0007438 \int V_{out} \, dt + 0.120 \qquad (1)$$

where, F: Force applied to the PVDF film.

$\int V_{out} \, dt$: Integrated voltage output from the PVDF film.

Details of the sensor development and calibration are presented in [23].

2.1. Zebrafish egg cell preparation

Zebrafish (Danio rerio) were selected as an animal model because of their easily accessible eggs, short generation time, high fecundity, rapid development, external fertilization and translucent embryos. Moreover they develop solid organ malignancies analogous to human tumors. Zebrafish were obtained from "Scientific Hatcheries" (Huntington Beach, CA) and were maintained under standard conditions. Adult female zebrafish were caught and immersed in a beaker containing 100 ml of aquarium water and 4.2 ml of 0.2 % tricaine solution for 5-10 seconds. After confirming anesthesia, fish were removed and
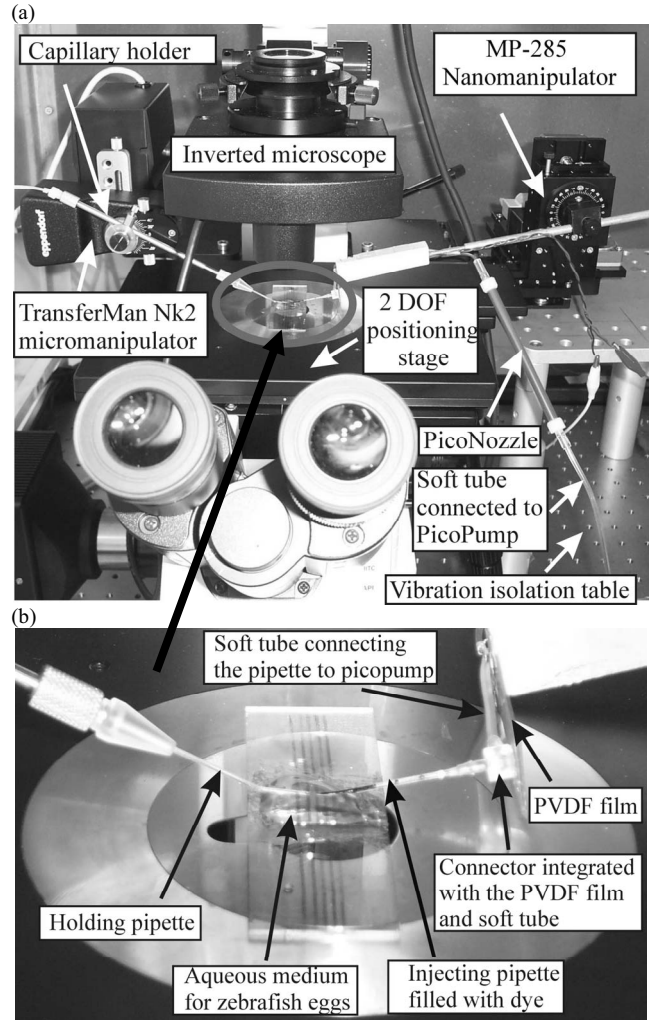

Fig. 1(a). The cell manipulation system.
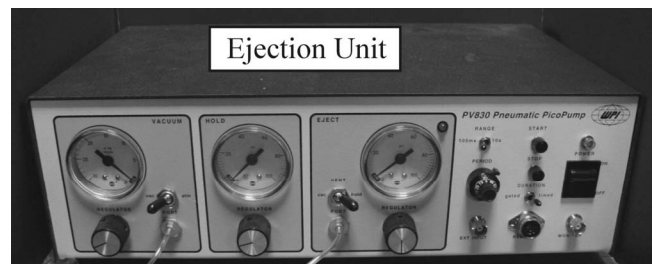Fig. 1(b). Magnified view of the system.

Fig. 2. The pneumatic PicoPump regulates air pressure for injecting dye into cell.

placed in a petri dish where eggs were expressed by gentle compression. Freshly harvested eggs were used for each component of the experiment. The diameter of the egg is approximately 600-700 μm.

2.2. Experimental Setup and Research Protocol for Cell Injection

We were interested in evaluating the role of force feedback in cell injection. The outcome of the injection process (success or

failure) was judged by injecting trepan blue dye in zebrafish egg cell. We created two different scenarios in our experiment: (a) **S_1:** the subject was prohibited to see the dye being injected and (b) **S_2:** the subject was allowed to see the dye being injected. In a practical situation, the first scenario (S_1) corresponds to injecting non-transparent cells whereby it is impossible to ascertain the presence of the dye (colored or colorless) injected in the cell and the second scenario (S_2) corresponds to injecting transparent cells (zebrafish eggs, for example) with a colored dye. Since we are working with only one type of egg, namely, zebrafish, which is transparent, we created experimental conditions for scenarios S_1 and S_2 by differentiating whether the subject cannot see (S_1) or can see (S_2) the injected colored dye within the cell.
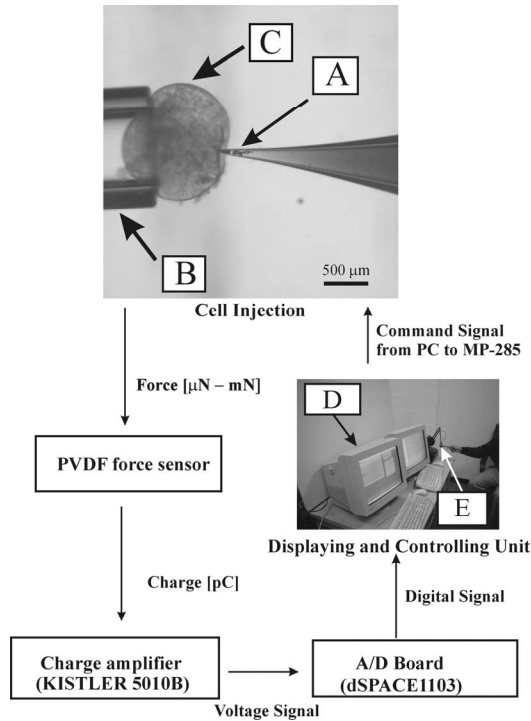

Fig. 3. The Cell injection system with vision and force feedback interface. A: Injecting pipette, B: Holding pipette, C: Zebrafish egg cell, D: Visual interface, E: PHANToM haptic interface.
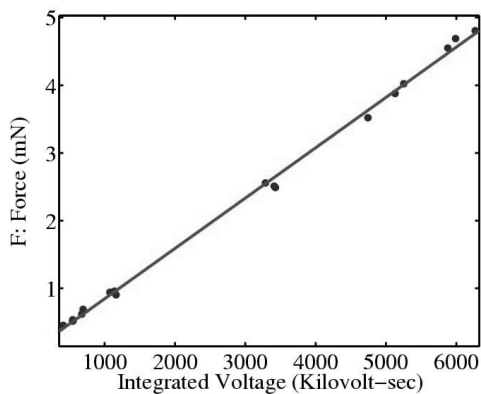

Fig. 4. Calibration curve showing a linear relationship between the force measured by the load cell and the integrated voltage from the PVDF film.

Since in conventional biomanipulation tasks, the cell may or may not be transparent, our experimental scenarios S_1 and S_2 described above cover the most general cases to study the effect of force feedback in biomanipulation tasks. We had 40 human subjects perform the experiments with vision (V) feedback alone and combined vision + force (V+F) feedback, with 20 subjects allocated for S_1 and 20 subjects allocated for S_2. All the subjects were non-surgeons having no previous experience with cell injection tasks. Each subject performed 5 trials with vision (V) feedback alone and 5 trials with vision + force (V+F) feedback. Details for both the (V) and (V+F) feedback protocol are presented below.

2.2.1. Vision (V) Feedback Protocol

The first part of the experiment consisted of only vision feedback to perform the cell injection task. The subject was able to view injecting pipette through the eyepiece of the inverted microscope. An operator applied suction and fixed the zebrafish egg cell. At this moment, center of the egg was not aligned with the tip of the injecting pipette. With the help of the joystick (TransferMan NK2) the subject controlled the movement of the holding pipette and aligned the egg with the tip of injecting pipette. The egg and the tip of injecting pipette were maintained at a fixed distance apart by the subject. For the first test, a practice session was given to familiarize the subject with the alignment task. The subject was able to view the alignment on a video screen and moved the injecting pipette forward with the help of computer controlled nanomanipulator. The subject observed the injecting pipette penetrating the egg membrane on a video screen and stopped the motion of injecting pipette when he/she was confident that pipette had penetrated the cell membrane. At this moment, dye was injected by the operator by depressing the foot pedal switch. The injection was deemed successful, when the dye remained inside the cell and the cell did not collapse on removing the pipette. Since the dye is blue in color, it was straight forward to determine if the dye remained in the cell after injection. The volume of dye injected was approximately 0.001 times the volume of the egg cell. Completion time for the injection task (including alignment task) was recorded. The process was repeated for five trials for each scenario S_1 and S_2. After each trial of S_1, the subject was asked to leave the console allowing the operator to inject dye in the absence of the subject.

2.2.2. Vision and Force (V + F) Feedback protocol

The second test was conducted by using both vision and force feedback to perform the cell injection task. This test was performed in the same way as the vision test, with the addition of force feedback. For this experiment the subject used the PHANToM (haptic interface device manufactured by Sensable Technologies, Inc.) by holding its stylus. The forces were amplified by a factor of 800. The direction of the force feedback was horizontal and was acting towards the subject. The operator controlled the movement of the injecting pipette with the help of computer controlled nanomanipulator. If the subject contacted the cell membrane and pressed against it, he/she would perceive an apparent increase in force followed by a drop in force when the membrane was punctured. A typical force profile for cell membrane penetration is shown in Fig. 5. During cell injection, the PVDF film was subjected to a force which increases with time until the cell membrane was punctured. After feeling the drop in force, the subject communicated to the operator to stop the motion
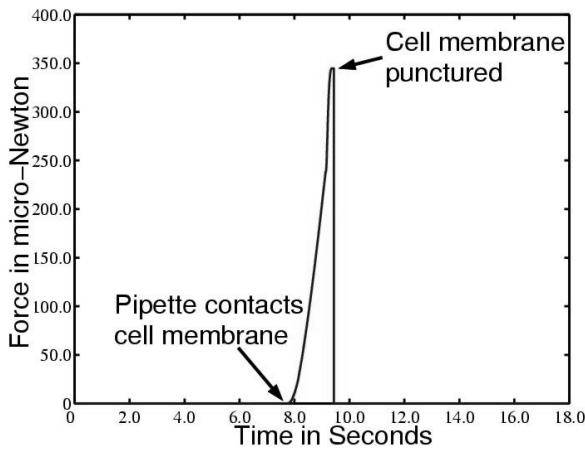
Fig. 5. Variation of force with time during membrane puncture of a zebrafish egg cell. The puncturing force was 350 μN.

Fig. 6. The percentage of successful cell injection for each individual using (V) and (V+F) feedback. (a) In S_1, the subject was prohibited to see the dye being injected. (b) In S_2, the subject was allowed to see the dye being injected.

of the injecting pipette. The operator injected the trepan blue dye after the subject confirmed that the cell membrane was punctured. The criterion mentioned in the vision test was used to judge the outcome of the injection process. Completion time for the injection task (including alignment task) was recorded. The process was repeated for five trials.

2.3. Data collection and analysis

The experiment was performed by 40 subjects (20 subjects for each scenario S_1 and S_2) for a total of 400 trials (5 trials each for V and V+F; hence 10 trials by each subject). The data were collected in a qualitative fashion with cell injections characterized as either "success" or "failure" and denoted by a value 1 or 0 respectively since trepan blue dye is easily observed under the microscope. The data were then analyzed using a non-parametric equivalent of the paired t test i.e. Wilcoxon test. The test generates a p value (probability) for the null hypothesis (H_0) and thus a probability for the research hypothesis (H_1) to be tested. The lower the p value, the smaller the probability for the null hypothesis to be true and consequently higher is the probability that there is a significant statistical difference between the data sets (or the research hypothesis H_1 is true). The level of significance (α value) for our statistical analysis was chosen to be 0.05, meaning that our research hypothesis would be considered true if p < α.

3. RESULTS

We performed the above experiments to test the validity of our research hypothesis, namely, providing vision + force feedback simultaneously leads to higher success rate in cell injection task than only vision feedback ((V + F) > V). The ">" sign denotes "is better than" in the hypothesis. Vision feedback alone was tested before combined vision + force feedback because of the presence of more than one sensory cue. If the above approach is not followed, the subjects may link the drop in force to the visual cue of cell membrane puncture and that may contribute to a learning effect. The trials were, thus, presented in the following order: vision feedback alone followed by vision + force feedback. As seen from Fig. 6, the outcome of cell injection with combined vision + force feedback is superior to vision feedback alone for each subject. The effect of learning is shown in Fig. 7.
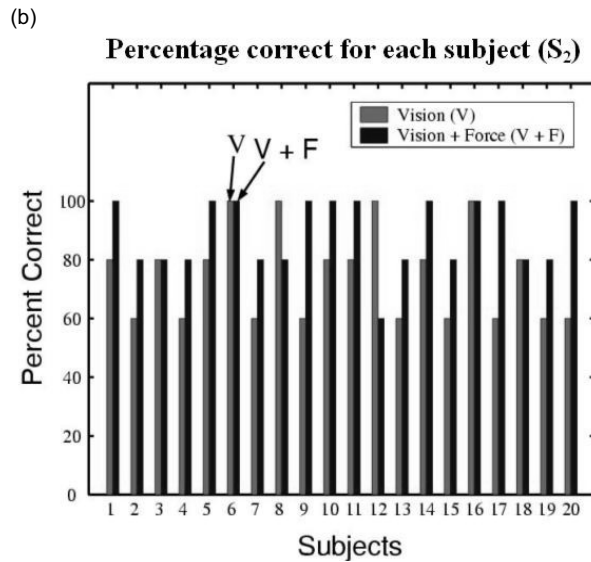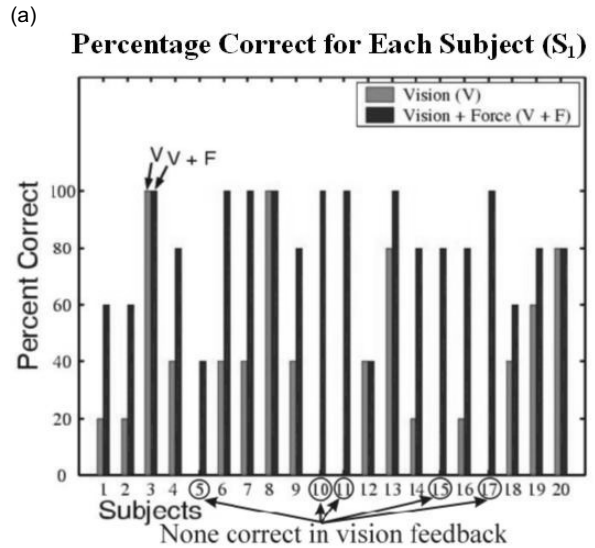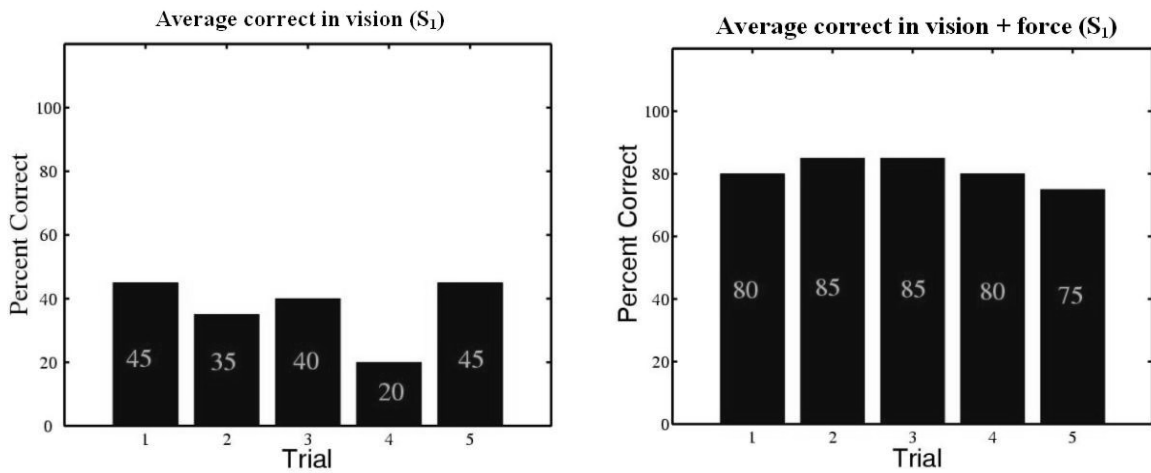
As observed from the figure, there is a significant learning effect using vision feedback in S_2 compared to using combined vision + force feedback. There was no learning effect observed in S_1 for both V and V + F feedback and the learning behavior of the subject using vision feedback in S_2 did not extend into V + F feedback. Thus, the better performance using V + F feedback compared to vision feedback alone is due to the addition of force feedback. Overall, the average success for S_1 and S_2 is shown in Fig. 8. Paired t test (Wilcoxon test) was performed to evaluate whether there was a significant difference in each trial between 2 data sets (V and V + F) for different scenarios S_1 and S_2. Table 1 shows the p value generated when comparing the data sets for each trial and average success, for scenarios S_1 and S_2. The p value was less than α value (0.05) for all trials in S_1 and for trials 1 & 2 in S_2. Thus comparing the 2 data sets (V & V+F) in S_1 for each trial and in S_2 for trials 1 & 2, there exists a probability of greater than 95%, that there was a significant difference between the two data sets.

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(a)

Average correct in vision (S$_1$)

Average correct in vision + force (S$_1$)

(b)

Average correct in vision (S$_2$)
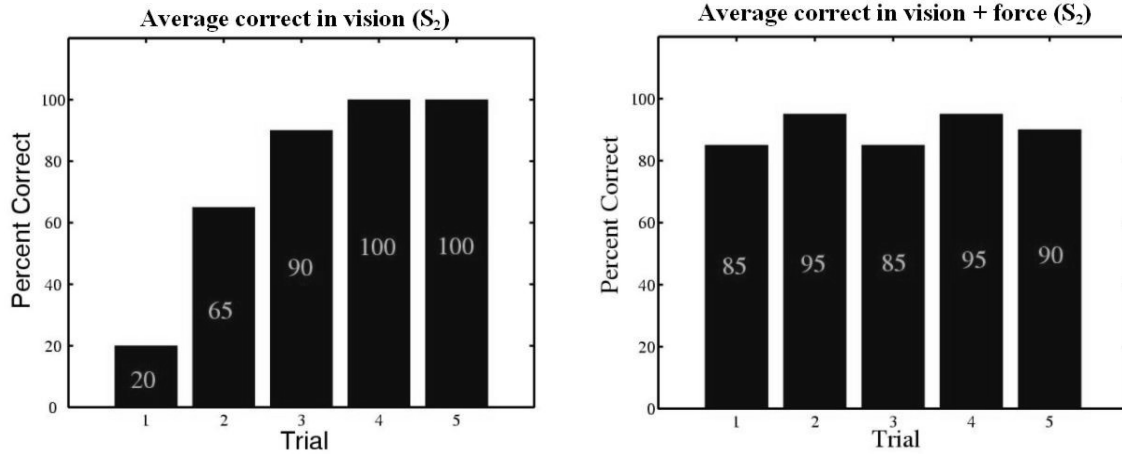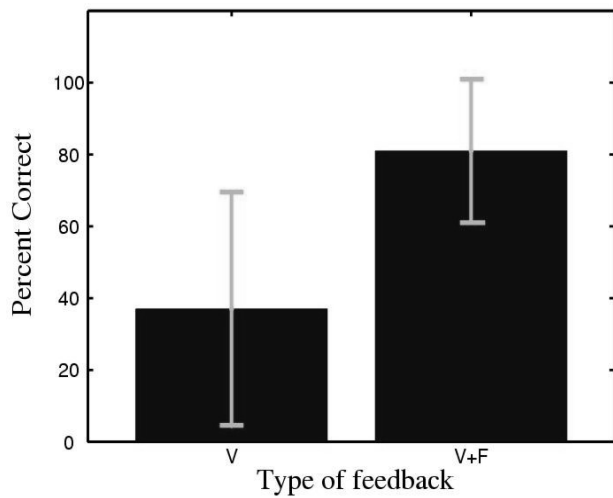
Average correct in vision + force (S$_2$)

Fig. 7. Percentage of successful injections for all five trials using only vision feedback and using combined vision + force feedback: (a) for S$_1$ (b) for S$_2$. Learning effect is clearly observed for vision feedback in S$_2$, as expected.
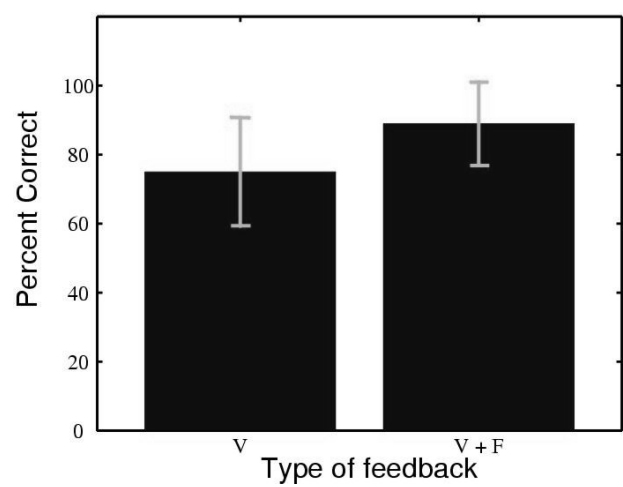
(a)

(b)

Fig. 8. In two experimental scenarios, S$_1$ and S$_2$, the percentage of successful cell injection for all 20 subjects.
(a) For S$_1$: Average success using only vision feedback (37%) and vision + force (V + F) feedback (81%).
(b) For S$_2$: Average success using only vision feedback (75%) and vision + force (V + F) feedback (89%).

Table 1. The p value generated when comparing the 2 data sets (V & V+F) for each trial and average success for: a) S_1 and b) S_2 (*indicates $p < \alpha$, where $\alpha = 0.05$)

a) S_1

Trials	p value
1	**0.017***
2	**0.001***
3	**0.0065***
4	**0.0005***
5	**0.030***
Average success	**< 0.0001***

b) S_2

Trials	p value
1	**0.0005***
2	**0.017***
3	0.32
4	0.15
5	0.07
Average success	**0.005***

There was no significant difference when comparing the data sets for trials 3, 4 & 5 in S_2, which shows that subjects had a learning effect. The p value obtained when comparing the average success for the 2 data sets for S_1 and S_2 was less than 0.0001 and equal to 0.005 respectively, leading to a probability of greater than 99.99 % that there was a significant difference between the data sets. Hence, there was a significant improvement in success rate using combined vision + force feedback compared to using vision feedback alone for S_1 as compared to S_2. Also there is a large standard deviation for vision feedback alone for S_1 as compared to S_2. Thus force feedback plays a major role in improving the success rate while injecting non transparent egg cells with a color/colorless dye or injecting transparent egg cells with colored material (trepan blue dye), but it cannot be ruled out that the subjects learnt and improved the success rate using vision feedback alone in S_2. Fig. 9 shows one of the injection tests with vision feedback alone which was unsuccessful and Fig. 10 shows one of the injection tests with combined vision and force feedback which was successful based on the research protocol outlined in section 2.2. In most of the unsuccessful cell injection tasks the subjects perceived that they had penetrated the cell membrane while in reality they had not. The range of pipette movement by the subject cannot be very large since that could lead to cell rupture or the pipette reaching the other end of the cell membrane. The obvious advantage of force feedback is the perceived drop in injection force after the membrane is punctured, which resulted in a successful cell injection task. The average completion time taken by each subject to perform experiments with V and V + F feedback for S_1 and S_2 scenarios is shown in Fig. 11. As seen from the figure there was no significant difference in the completion time for each subject in V and V+F feedback. However there was a significant variation in completion time across subjects.

4. CONCLUSION

We have developed an automated cell injection system with force feedback capability along with visual display. The force sensing system is capable of measuring forces in the μN range. Our results confirm that subjects had a higher degree of success in injecting the desired material (trepan blue dye) into the cell with simultaneous vision + force feedback compared with vision feedback alone. Overall, considering all 40 subjects, the research

hypothesis was validated through our experimental results. Statistical analysis proved that there is a significant difference between the 2 data sets (V and V+F). This system has potential application in injecting genetic material into cells to create transgenic organisms with high success rate. In our future work, we plan to develop microelectromechanical systems (MEMS) based cell injection system to hold the cell and fixate the nucleus so that the genetic material can be injected directly into the nucleus for efficient transgenesis.
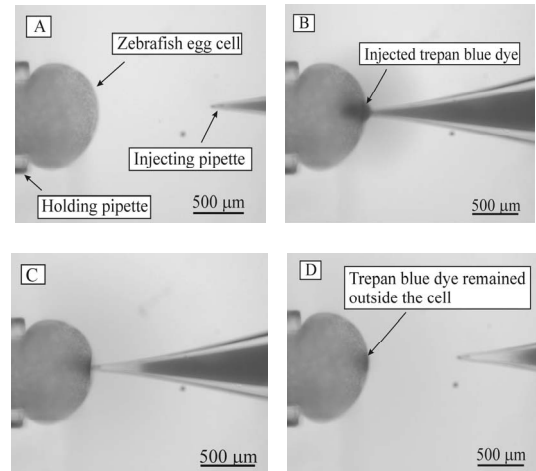

Fig. 9. Example of an unsuccessful injection of zebrafish egg cell with vision feedback alone.
(A) The injecting pipette approaching the cell.
(B) The subject stopped the forward motion of the pipette being certain that the cell membrane is punctured, at this moment trepan blue dye was injected into the cell.
(C) The pipette being withdrawn.
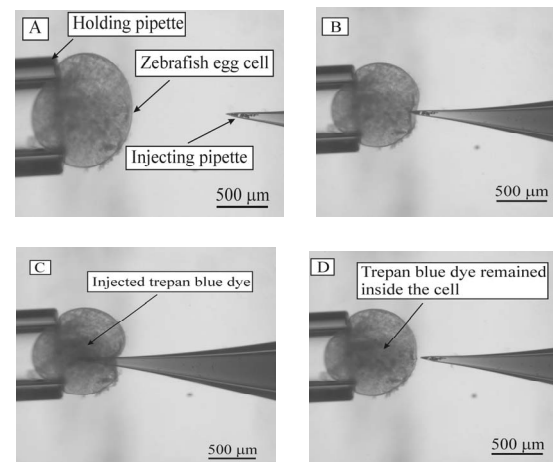(D) The trepan blue dye remained outside the cell.

Fig. 10. Example of a successful injection of zebrafish egg cell with vision and force feedback.
(A) The injecting pipette approaching the cell.
(B) The injecting pipette in contact with the cell membrane.
(C) The subject stopped the forward motion of the pipette when perceiving an apparent drop in force; at this moment trepan blue dye was injected into the cell.
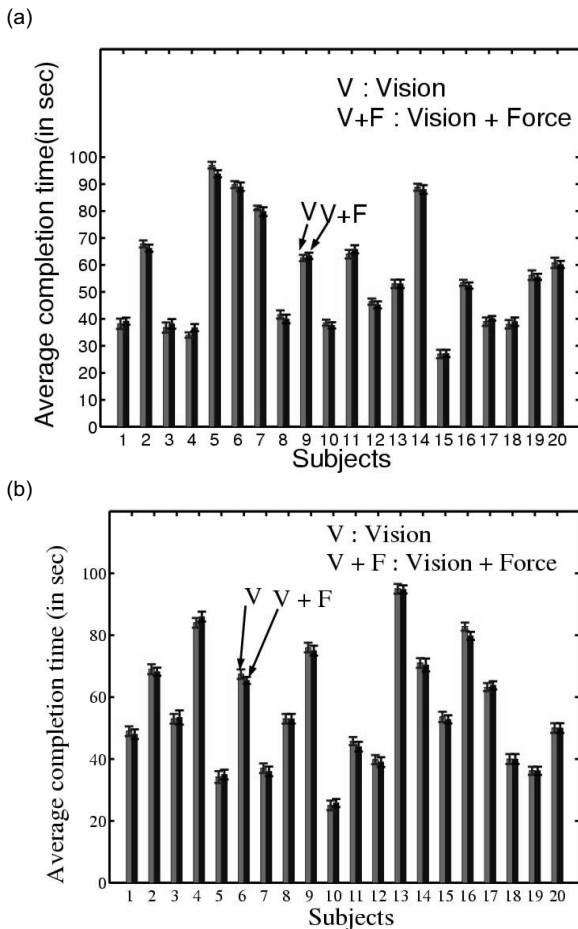(D) The trepan blue dye remained inside the cell.

(a)

(b)

Fig. 11. The average completion time taken by each subject to perform cell injection with vision (V) feedback and vision + force (V+F) feedback for: a) S_1 and b) S_2.

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