

Force Feedback Interface for Cell Injection*

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Abstract

Manual pronuclei injection and intracytoplasmic sperm injection (ICSI) requires long training and has low success rates primarily due to poor control over the injection force. Consequently, there is a need for quantification of forces during biological cell injection and for an automated cell injection system, which can provide force feedback to the operator improving the success rate of the injection task. We have developed a force feedback interface, which has the capability of measuring forces in the range of μN - mN and provide a haptic display of the cell injection forces. The force sensor has been integrated with the biomanipulation system to detect forces in real time. Experiments were performed on two different varieties of egg cells to demonstrate the success in measuring forces in the range in μN - mN . Our experimental results indicate the cell puncturing forces were consistent and the operator was able to feel the cell injection forces.

1. Introduction

Bio-surgery on individual cells, ordered cell arrays or aggregates of cells will be the platform for the next generation of genetic manipulation. Individual manipulation of biological cells referred to as “biomanipulation” is being extensively used in in-vitro fertilization and transgenics [1]. Manipulations may include correctional intervention during early stages of fetal development, treatment of diseased areas in the body without the required resection or organ transplantation, and for a wide range of other molecular and intracellular nanotechnology applications. To achieve bio-surgery reliably and accurately, operators must have accurate haptic (“feel”) and visual feedback from the cell as intracellular injection is conducted. A haptic and visual feedback system can be used to manipulate an individual cell or array of cells to standardize outcomes of cellular

surgical procedures. Further advancement of this technology can be reliable and accurate gene injection at specific target sites within the cell or in the nucleus.

Until now, visual feedback has primarily been used to perform cell injection tasks. A suction pipette holds the cell while the operator uses a second pipette to inject the cell. This is a bi-manipulation task. However, visual feedback alone with cell suction (which can cause cell damage) is not sufficient. Repeated studies indicate that skilled operators with about a year of training only have about 15% success rate during the entire transgenic task (gene injection into the cell). Secondly, the process is not repeatable and accurate. Most importantly, regional or organ specific delivery within an embryo or fetus is virtually impossible with manual techniques. Hence, an accurate and reliable cell injection system is needed.

Few researchers have developed Piezo actuators for cell manipulation, [2-14] which offers highly repeatable motion and increases the chances of the oocytes survival rate. A variety of piezo actuators have been developed and implemented to develop a multi-microrobot manipulation system prototype such as MiCRoN [15] to handle nm- μm sized objects. Various control schemes had been proposed for improving the displacement behavior of piezoelectric actuators [16-24]. Calibration of micromanipulators had been proposed to increase the positioning accuracy [25-27]. Significant research had been done to develop and control micromanipulation systems [28, 29]. Other biomanipulation techniques involve using optic, electric, magnetic or acoustic energy [30-34]. It has been reported in the literature that the laser beams used in laser trapping can cause damage to the cell. Also, electric field based manipulation provides insufficient holding force. Zhou et al developed a cantilever-based optical sensor, which measures forces in the range of nN [35]. The limitations involved in such a technique is that a complex transmitter-receiver set up is required and the photodiode can detect only a small range of deflection and the force measurement is inaccurate due to reflection and refraction of the transmitted light through aqueous medium where the biological cells survive [36]. The disadvantages associated with the non-contact manipulation methods have motivated us to explore contact biomanipulation techniques. There had been focus on different control strategies to develop a visually servoid microrobotic

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system [37-45]. Cell detection algorithms had been developed which support the automatic cell manipulation system [46-50]. The mentioned techniques provide only visual feedback to the operator and consequently the cell viability outcomes from such techniques may not provide a significant improvement over existing methods. Combining force feedback with vision can lead to better cell injection outcomes since the operator can feel and see the cell injection process. This will allow the successful deposition of the material (DNA or sperm) into the egg cell, causing no damage to the cell.

Bilateral control of micromanipulators has also been discussed in the literature [51-53]. Preliminary work had been done to provide force feedback in micromanipulation [54, 55]. But the experiments were performed on rigid object 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 [56, 57]. The measurement of forces provides a basis to develop a mechanical model for the cell, which will quantify its mechanical properties [58]. The model can be used in a virtual simulator to provide training for the operator before they perform the cell injection on actual cells.

In this paper we have developed a force feedback interface to provide force feedback to the user as the cell is injected. As a proof-of-concept of our initial work, we performed experiments on salmon and flying fish egg cells. The paper consists of 4 sections. In section II, we present the materials and methods used in our work. In section III, we present the results from our experimental work on two types of egg cells. Finally in section IV, we make some concluding remarks and the directions for future work.

2. Materials and Methods

PVDF (Polyvinylidene fluoride) piezoelectric polymer film is used to develop the force sensor for measuring the cell injection forces. The PVDF film is ideal for our application because of excellent sensitivity, high compliance and high signal to noise ratio [59-61]. Fukuda et al [62] and Lin et al [63] developed a force sensor based on strain gauge principle. The strain gauge being less sensitive than the piezo film is not able to display the true force in the μN - mN range. Figure 1 shows the overall setup on the biomanipulation side, which consists of a Nanomanipulator (Model: MP-285, manufactured by Sutter, Inc.) holding the PVDF force sensor and the pipette holding apparatus. The Nanomanipulator has three degrees of freedom in x, y and z direction and an additional fourth degree of freedom for the diagonal advancement of the pipette in the XZ plane (refer to Figure 1). The travel range is 25mm in all three axes. The lowest resolution is $0.02 \mu\text{m}/\text{step}$ and highest resolution is $40\text{nm}/\text{step}$. The glass micropipette is integrated to the PVDF film

(Thickness: $28\mu\text{m}$, Model: LDT1-028K of MSI, Inc.) with the help of a connector as shown in the Figure 2. This setup allows the easy removal and replacement of the micropipette if the tip of it gets damaged during micromanipulation ($5\mu\text{m ID}$).

Theoretical Model: A theoretical model for the PVDF film is developed using the methodology proposed in [64] with some modifications. The following parameters will be used in the analysis:

W: width of PVDF Film

h: thickness of PVDF film

L: length of PVDF Film

A: surface Area ($L * W$)

a: cross-sectional area ($W * h$)

$Q(t)$: charge produced (PVDF Film)

$I(t)$: Current produced (PVDF film)

$V(t)$: voltage across the PVDF Film

R_p : resistance of PVDF Film

C_p : Capacitance of the PVDF Film

$F(t)$: Contact force (such as cell injection force)

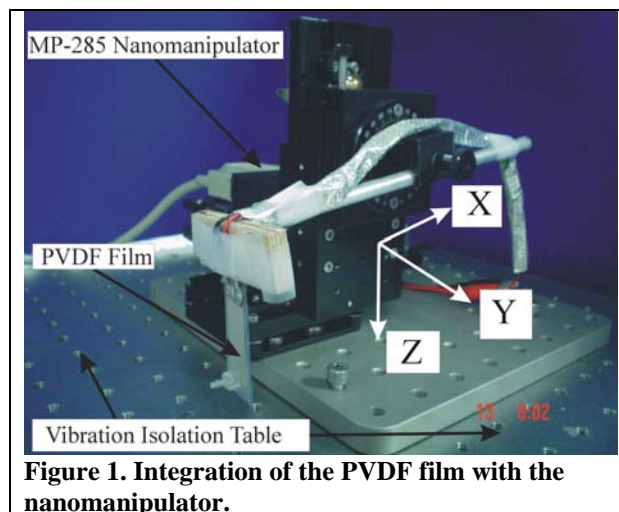


Figure 1. Integration of the PVDF film with the nanomanipulator.

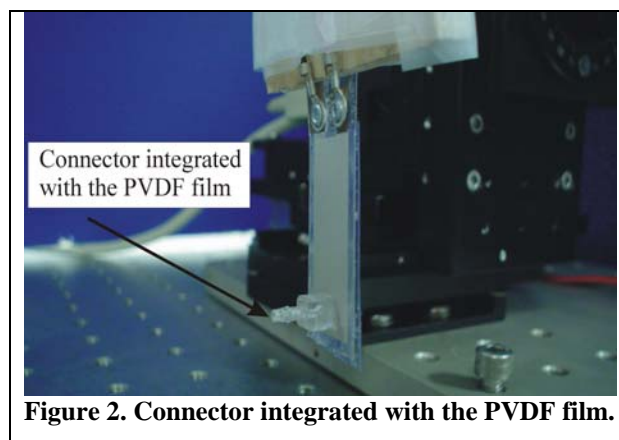


Figure 2. Connector integrated with the PVDF film.

I_{xx} : inertial moment of cross sectional area, a
 $\sigma(x,t)$: unit stress
 d_{31} : piezoelectrical coefficient of PVDF film
 ϵ_{33}^T : dielectrical factor of the PVDF film
 $E_3(t)$: Electrical field factor of the PVDF film
 $D_3(x,t)$: Unit polarization factor of PVDF film
 From the theory of mechanics of materials, for a cantilever beam (see Figure 3):

$$\sigma(x,t) = \frac{F(t)x \frac{h}{2}}{I_{xx}} \quad (1)$$

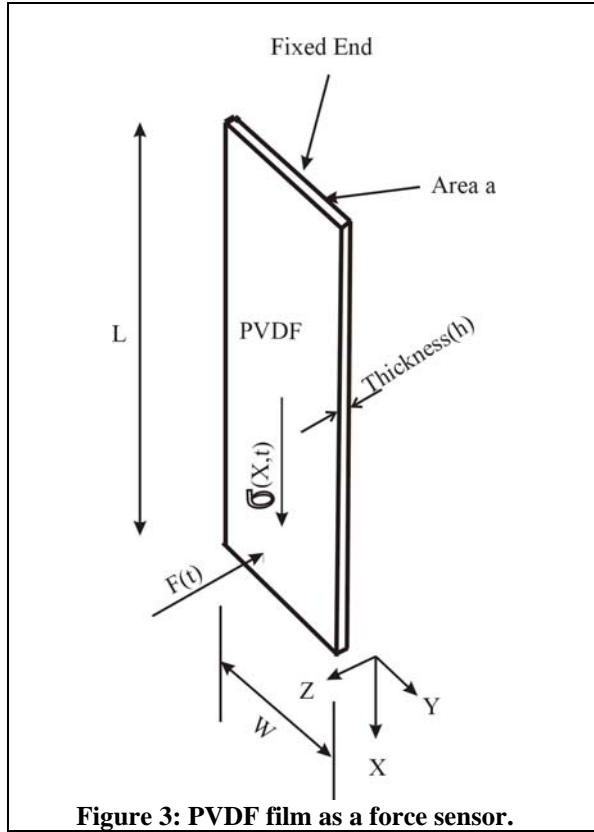


Figure 3: PVDF film as a force sensor.

Equation (1) is valid under the assumption that the neutral axis of the bending deflection of beam is assumed to pass through the centroid of cross sectional area. The total charge generated on the surface of the PVDF film due to the stress, $\sigma(x,t)$ is given by:

$$Q(t) = \int_0^L d_{31}\sigma(x,t) + \epsilon_{33}^T E_3(t)A \quad (2)$$

Modeling the PVDF film as a charge source in parallel with a capacitor C_p , the output current $I(t)$ is given by:

$$I(t) = \dot{Q}(t) = C_p \dot{V}(t) \quad (3)$$

As the resistance R_p , of the PVDF film is very high (typically of the order of 10^{13} ohms), it is not considered

in our model unlike the model presented in. [64] The Electrical field $E_3(t)$ is given by:

$$E_3(t) = -\frac{dV(t)}{dh} \quad (4)$$

Assuming a uniform electric field over the cross-sectional thickness, h , we can write $E_3(t)$ as:

$$E_3(t) = -\frac{V(t)}{h} \quad (5)$$

From the above equations (1-5), the following equation is derived depicting the relationship between the generated voltage across the PVDF film and the contact force, F :

$$V(t) = BF(t) \quad \text{where } B = \frac{Ad_{31}hL}{8I_{xx}C_p} \quad (6)$$

Since the voltage output from the charge amplifier is proportional to the current input (produced by the external force applied to the PVDF film), we have:

$$\frac{V_{out}}{I} = G = \text{Constant} \quad (7)$$

Combining equations (3), (6), and (7), we get:

$$F = \frac{1}{GBC_p} \int V_{out} dt \quad (8)$$

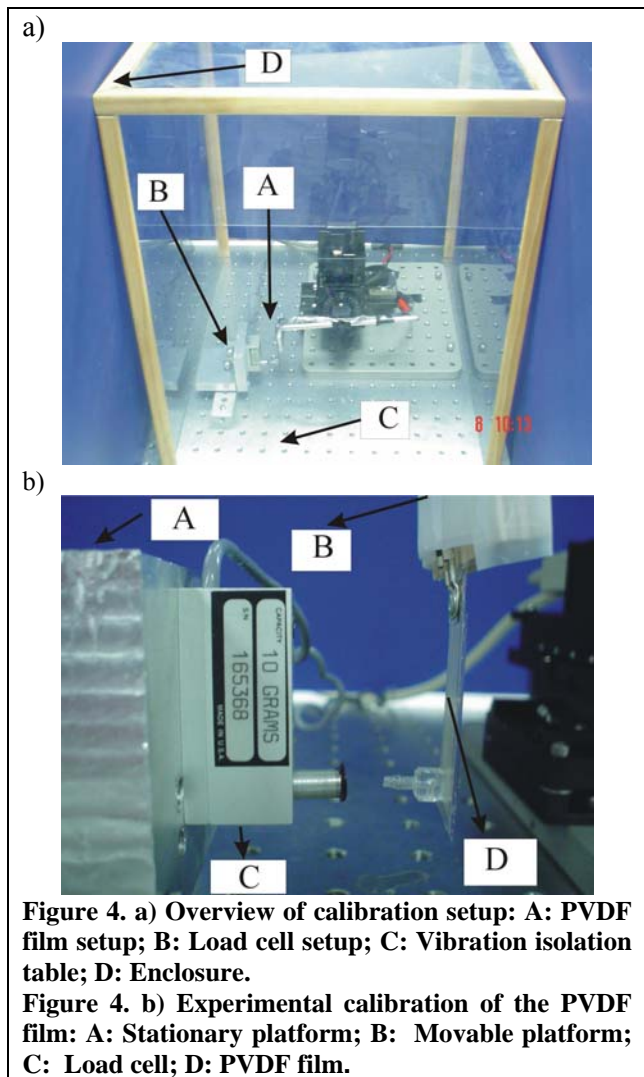
The above equation shows that there exists a linear relationship between the force applied to the PVDF film and the corresponding integral of the output voltage from the charge amplifier.

Experimental calibration: The PVDF Film is calibrated with the load cell (Model: GSO-10 of Transducer technology Inc., Maximum measurement range: 98.1mN and accuracy of 50μN). A vibration isolation table is used to remove ground vibrations and an enclosure is used to cover the whole calibration setup to eliminate the interference of acoustic waves on the output voltage from the PVDF film (since it has been observed to be highly sensitive). The load cell is mounted on a stationary platform and the PVDF film is mounted on the MP-285 Nanomanipulator. The experimental calibration setup is shown in Figure 4.

During calibration, the PVDF approaches the load cell and the connector contacts the load cell, which leads to charge generation across the PVDF film. This charge is fed to a charge amplifier (Model: 5010B, Kistler Instrument) to get an amplified voltage signal, which is measured, through a data acquisition board (model: dSpace 1103).

As the connector contacts the load cell, there is an output voltage from the charge amplifier and a corresponding force output from the load cell. The peak of the force signal corresponds to the peak of the integrated

voltage signal from the PVDF film as shown in Figure 5. The connector contacts the load cell at different velocities and range of distances, so the PVDF film bends depending on the distance traversed by the nanomanipulator. Due to



different velocities and range of distances traveled by the PVDF film, the strain developed in the PVDF film will result in a generation of different voltage signals from the amplifier. We performed several such measurements to derive the experimental calibration curve shown in Figure 6. The experimental calibration curve is given by:

$$F = 0.0007438 \int V_{out} + 0.1020 \quad (9)$$

which shows that the relationship between the force measured by the load cell and the integral of the voltage output from the charge amplified is linear.

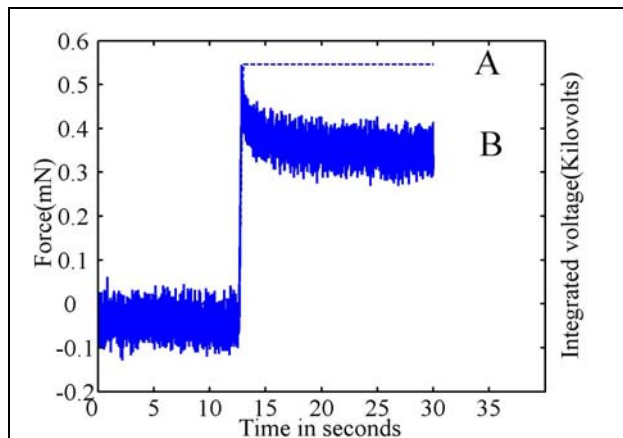


Figure 5. Plot showing the peaks of the force profile and the integrated voltage for a typical calibration measurement: A: The integrated voltage signal from the PVDF film in kilovolts; B: The force signal from the load cell in mN.

A comparison of equations (8) and (9) shows that there is a linear relationship between the applied force and the corresponding integral of the voltage output from the charge amplifier. The offset in the experimental calibration curve could possibly be due to pyroelectric effects (they have not been considered in the derivation) as well the effect of acoustic waves and ground vibration (even though a vibration isolation table and an enclosure were used). The ground vibrations and acoustic waves cannot be completely eliminated from the experimental setup.

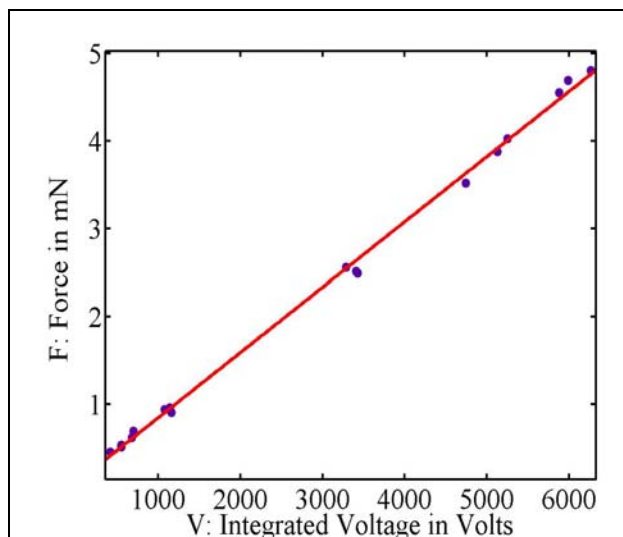
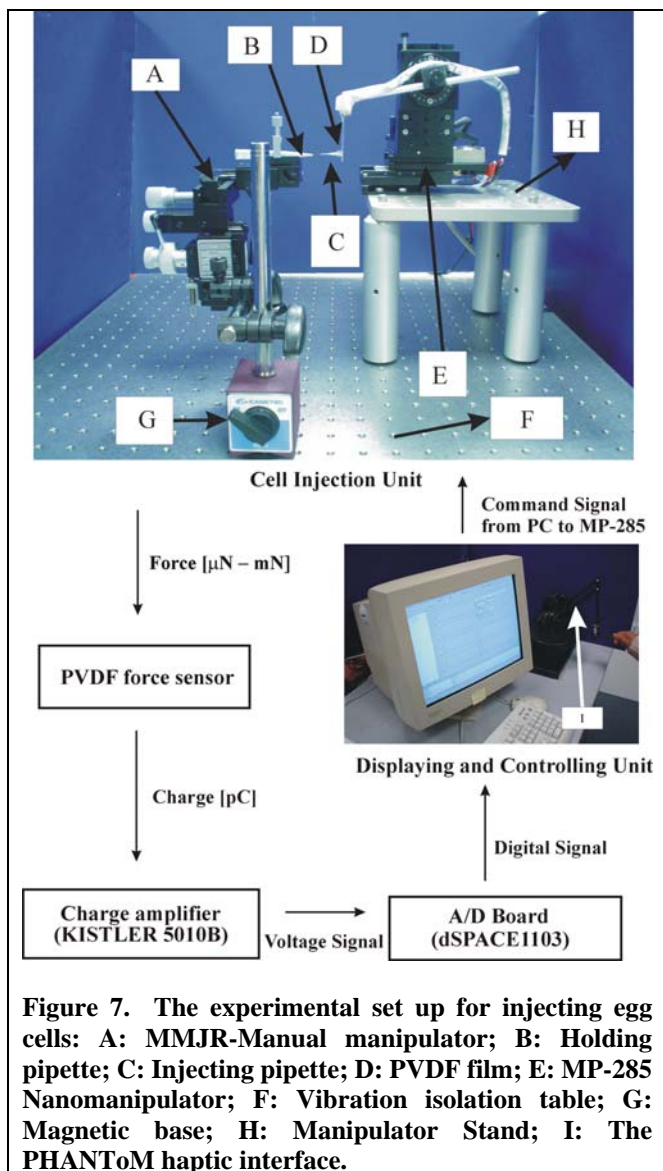


Figure 6. Calibration curve showing a linear relationship between the force measured by the load cell and the integrated voltage from the PVDF film.

3. Results

To test our force feedback interface for cell injection, we performed several cell injections on two different types of egg cells, namely, salmon fish egg cell and flying fish egg cell. The experimental setup consisted of the PVDF film, nanomanipulator, micropipette (Tip ID: 5 μ m) inserted into the hollow connector, and the PHANToM haptic interface device. A plastic micropipette holds the



egg cells. The holding micropipette is mounted on to the MMJR, manual manipulator (manufactured by WPI, Inc.) which has three degrees of freedom in x, y, and z-direction. The travel range is 37mm in X-axis, 20 mm in Y-axis and 25mm in Z-axis. The resolution is 0.1 mm in all three axes. The cell manipulation system has a total of

six degrees of freedom (3 DOF for holding pipette and 3 DOF freedom for the injection pipette) as shown in Figure 7. A magnetic base is used for the positioning and holding of the manual manipulator. The whole set up is mounted on a vibration isolation table and covered by an enclosure (not shown in figure 7). The egg cells are sucked inside the holding pipette, which facilitate the penetration of the injection pipette into the egg cell. The diameter of salmon fish egg cell is around 4mm-6mm and the diameter of the flying fish egg cell is around 700 μ m – 1mm as shown in Figure 8. The Velocity of the manipulator during injection is 120 μ m/sec. Before performing the experiment the holding pipette is aligned with the injection pipette, which facilitates the injection pipette to penetrate into the egg. The injecting and holding pipette are aligned by controlling the 6DOF of the cell manipulation system in such a manner that the tip of the injecting pipette contacts the center of the egg cell. While performing the experiment the injection pipette is moved towards the egg cell with the help MP-285 nanomanipulator, where as the manual manipulator is held stationary. To track down the pipette tip it is filled with a dye as shown in figure 9. Visible light is used for illumination. Using light of different wavelengths, in particular lower wavelengths, may cause some physical damage to the cell. As the egg cells are transparent in nature, the dark colored dye can be seen when the pipette penetrates the egg cell as shown in figures 9 and 10. There is no noticeable effect of the dye on the egg cells (non-living). Force feedback during cell injection is achieved by in real time by using the PHANToM haptic interface device. Though the PHANToM is capable of providing force feedback along three principal directions, in our experimental setup, the force was felt only in the direction opposite to the motion of the pipette (see Figure 11). The forces measured during cell injection are amplified and displayed to the user in real-time. As a result, the user can perceive the cell injection as an apparent drop in injection force after puncture (please see the results below).

The experiments were performed on 10 samples of each egg cell. From figure 12, it can be observed that there is a gradual increase in the force prior to puncture of the membrane. The maximum force is recorded when the cell membrane is punctured. From Figure 12, the puncturing force for one of the flying fish egg cell was observed to be 1.69mN. Based on 10 different samples of the flying fish egg cell, the average puncturing force was found to be 1.6057mN with a standard deviation of 0.33mN. The puncturing force values obtained for injecting 10 flying fish egg cells is shown in Figure 13.

During each of these cell injections, force feedback was provided to the user through the PHANToM and the user was able to discern when the pipette penetrated the egg cell. Similarly the typical force profile obtained for puncturing the membrane of the salmon fish egg cell is

shown in Figure 14. The puncturing force for one of the salmon fish egg cell was observed to be 2.38mN. In all the trials, we observed that the slope of the force vs. time curve was less steep for the salmon fish egg cell compared to the flying fish egg cell. This indicated that the salmon fish egg cell underwent more deformation than flying fish egg cell, before its membrane was punctured. The average force for puncturing the salmon fish egg cell for 10 different samples was 2.2694mN with a standard deviation

of 0.41mN. The puncturing force values obtained for injecting 10 salmon fish egg cells are shown in Figure 15. In all these experiments the measured forces were scaled by a factor of 200 when they were displayed to the user.

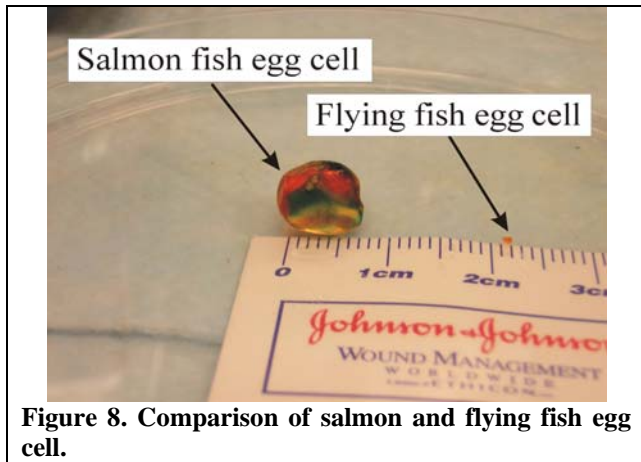


Figure 8. Comparison of salmon and flying fish egg cell.

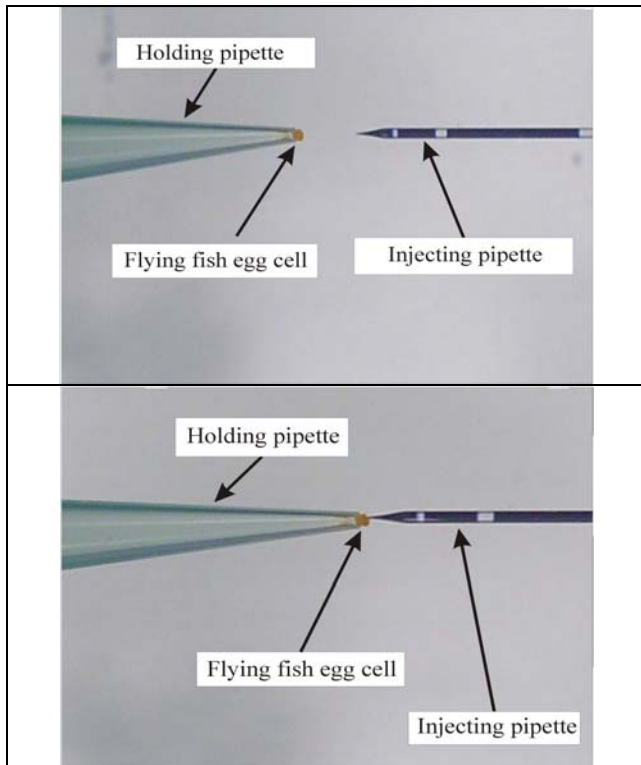


Figure 9. The figure on the top shows the injecting pipette approaching the flying fish egg cell and the figure on the bottom shows the injecting pipette penetrating into the egg cell.

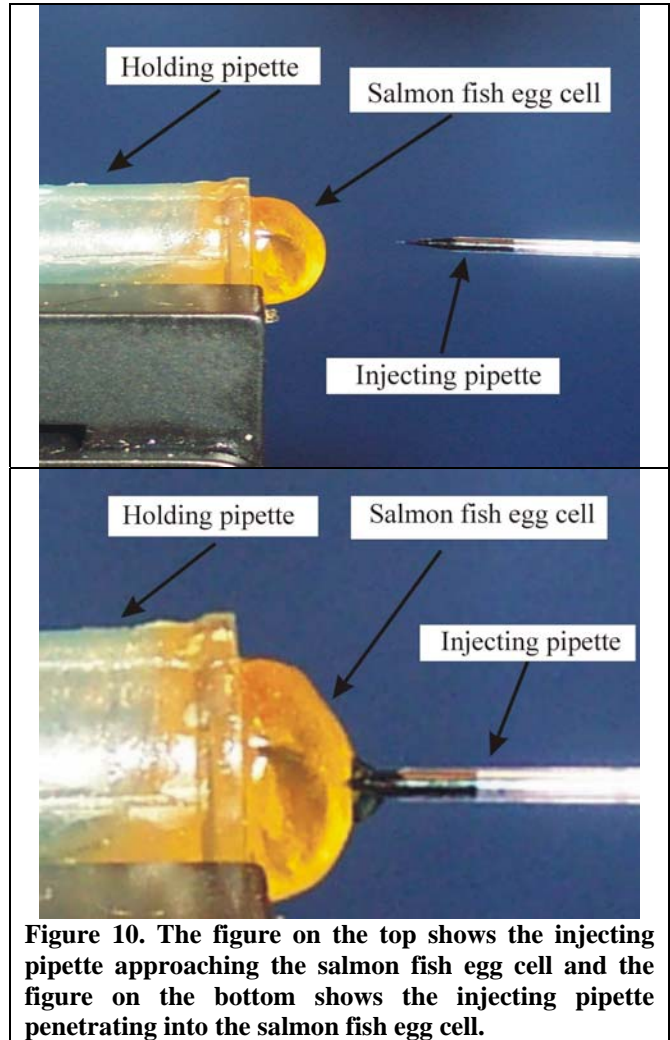


Figure 10. The figure on the top shows the injecting pipette approaching the salmon fish egg cell and the figure on the bottom shows the injecting pipette penetrating into the salmon fish egg cell.



Figure 11. Force feedback is achieved in real time through the PHANTOM.

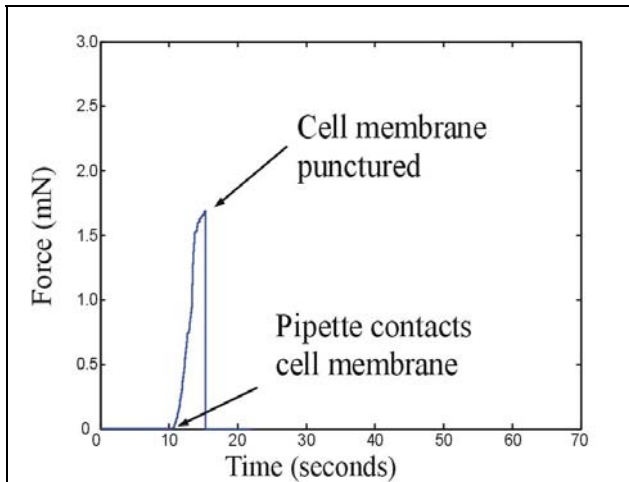


Figure 12. Variation of force with time during membrane puncture of a flying fish egg cell. The puncturing force was 1.69 mN.

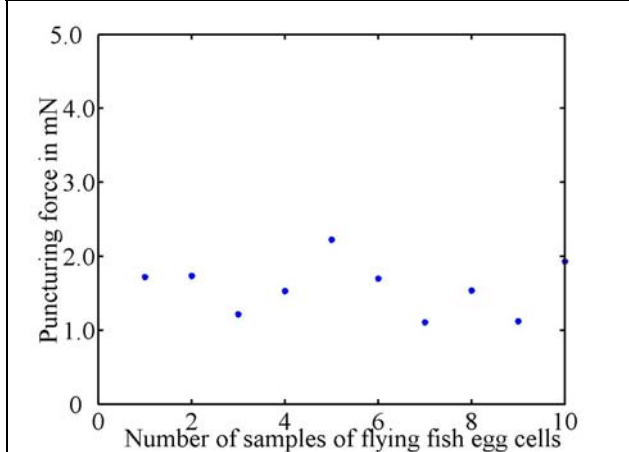


Figure 13. Variation of the puncturing force for 10 samples of flying fish egg cells. The average puncturing force was 1.6057mN.

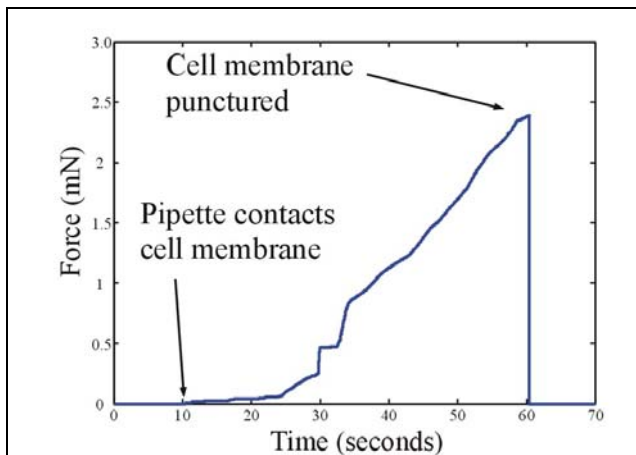


Figure 14. Variation of force with time during membrane puncture of a salmon fish egg cell. The puncturing force is 2.38 mN.

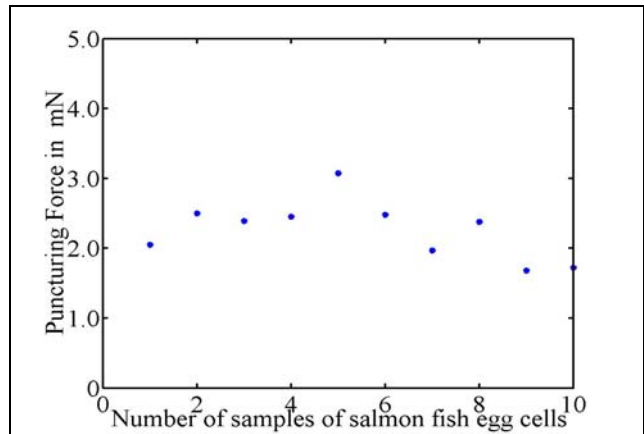


Figure 15. Variation of the puncturing force for 10 samples of salmon fish egg cells. The average puncturing force was 2.2694mN.

4. Conclusion

This paper describes a force feedback interface for reflecting forces to the user during cell membrane puncturing tasks. The force sensing system is capable of measuring forces in the μN – mN range. The successful implementation and calibration of the force sensor has been presented in detail. The average force values obtained for puncturing the outer membrane of flying fish egg cells and salmon fish egg cells was 1.6057mN and 2.2694mN respectively. During all membrane puncture tasks the user was clearly able to discern when the membrane was punctured through a rapid drop in the force felt through the PHANToM. The membrane puncture forces and the history of the force change prior to puncture can be used to develop cell models. However, the work presented in this paper cannot be directly applied to puncture smaller cells in the range of 50-100 μm diameter. In our future work, we plan to use the PVDF based force sensing system to effectively calibrate a vision-based force sensing system to quantify and display cell injection forces. Also, human factors studies comparing automated (force and vision feedback) vs. manual (only vision feedback) cell injection to improve the cell viability after injection will be the natural future direction of this research.

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