# Simple Biomanipulation Tasks with "Steady Hand" Cooperative Manipulator

Ankur Kapoor[1], Rajesh Kumar[2], and Russell H. Taylor[1]

[1] Johns Hopkins University, Baltimore, MD
{kapoor,rht}@cs.jhu.edu
[2] Foster-Miller, Inc. Waltham, MA
rkumar@foster-miller.com

**Abstract.** This paper reports preliminary experiments in the development of our Augmented Micromanipulation System using the JHU "Steady Hand" cooperative robot system to augment single cell manipulation tasks. The need for robotic augmentation of biomanipulatuon tasks is discussed. The JHU "Steady-Hand" robot configuration for cell manipulation is reported. Augmentation strategies for stable cooperative insertion of a micropipette in a mouse embryo are developed and preliminary experiments validating these strategies are presented. These preliminary experiments demonstrate promise of cooperative robotic augmentation in single cell manipulation tasks.

## 1 Introduction

Bio-manipulation tasks find wide applications in transgenic, biomedical and pharmaceutical research. Consider common biomedical laboratory tasks such as manipulating cells in a cell culture, or injecting genetic material in a cell using a micropipette. Applications of these tasks are research on transgenic organisms and IVF (*in-vitro* fertilization). For example, transgenic mice are constructed by injecting cloned DNA into fertilized mouse eggs. The eggs that survive the injection and continue to the two-cell stage after overnight incubation in culture are then implanted in foster females to develop to term. The mouse pups are then tested for transgenic status. There are several factors that affect the success rates including the purity and concentration of the DNA construct to be injected, human factors, and experimental factors such as injection accuracy and successful implantation. Published work [1] and an informal survey of several dedicated facilities performing cell injections for various purposes indicated a marginal 40%-70% survival rates for only cell microinjection (the success rate for entire transgenic task is much lower, only 1%-4%). Large variance due to human factors was also reported.

These micrometer scale laboratory biomanipulation tasks are currently performed with the following setup: a) micromanipulators for positioning and insertion of the micropipette, combined with b) stereo microscopes with high magnification and c) tools and fixtures to provide rigid fixation and damping of any vibrations. Even with this sophisticated equipment and trained operators accuracy and success rates of these tasks are marginal. Examples of these tasks include manipulation of individual cells, and injection of genetic material into cells. Non-contact manipulation methods such

R.E. Ellis and T.M. Peters (Eds.): MICCAI 2003, LNCS 2878, pp. 141–148, 2003.

as laser trapping are not suitable for these tasks since they also involve integral contact portions. Current joystick driven systems provide only visual feedback to the user.

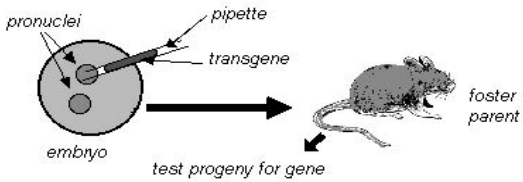


**Fig. 1.** Transgenic mouse model process

Our Augmented Micromanipulation System (AMS) research aims to take advantage of the precise manipulation capabilities of a cooperative robot, the analytical abilities of a computer, and the intelligence of a human. This initial work presents the first task (pronuclear microinjection) of injecting genetic material into a one day old mouse embryo that was used to validate the AMS concept. The scope of this preliminary work was limited to performing cooperative microinjection and establishing feasibility of a system that provides hands-on, flexible and intuitive means of performing biomanipulation tasks.

A prototype configured around the JHU "Steady Hand" [2] was used for this preliminary work. In the "Steady Hand" paradigm, the user shares the control of the tool with the robot, and receives an amplified feedback from the robot for the forces sensed by the tool tip. Since the user directly manipulates the tools, there may be added kinesthetic benefits from hand/eye coordination for biomanipulation tasks similar to larger-scale manipulation.

### 1. 1 Related Work

This preliminary research used the pronuclear microinjection of mouse embryos (Figure 1) as the example task for validation experiments. Prior published work also cites the utility of using robots for performing this task. Prior work primarily focuses on using teleoperated manipulators, in combination with vision methods to improve guidance, and automating portions of this task. The most relevant is work of Su and Nelson [3], and Codourey et al [4]. Su and Nelson present a custom micromanipulator for teleoperated microinjection. They use custom fixtures created to hold the cells in place during the process for easier operation and report successful autonomous injection into five embryos. Some analogies from previous work with surgical tasks [5-7] may apply here as well. Augmentation of biomanipulation tasks requires navigational assistance in addition to precise motion. Computer vision based methods have often been used to detect targets and augment robotic control [3,4,8,9], in particular for locating the embryo, and controlling the micromanipulator for the selected task.

## 2 Methods

The pronuclear microinjection task was observed as performed by trained users, and conventional operations were analyzed. This task involves the following steps, 1) transfer of 20-30 eggs into the injection chamber (in appropriate medium), 2) selection and fixation of each egg onto the end of the holding pipette, 3) injection of DNA solution into the (male) pronucleus of the egg. The injection is performed by piercing

the membrane of each egg with a single sharp motion. The angle of the needle should be close to perpendicular to the membrane surface (rather than glancing) to avoid tearing the plasma membrane. The pipette is withdrawn more slowly to avoid any further damage to the membrane.), and 4) removal of the injected egg to a culture and incubation at appropriate temperatures.

The mouse embryo is typically 50-100μm, and the positioning requirements are typically in micrometers. The interaction forces are typically in micro Newtons, too small to be sensed naturally by humans. Another concern during microinjection is damaging the cell during insertion or removal of the injection needle and during fixation of the cell to the holding pipette. The injection can cause considerable damage to the cell membrane leading to cell death. Transgenic organisms are produced in batches, and a large number of injections need to be performed within a limited amount of time, so efficient execution of this task is very important. Extensive user training is required to achieve proficiency and there is significant variability in the outcome.

The basic task outline above clearly suggests the use of an augmented robot system to enhance human capabilities. The outline also suggests that different positions, velocities and force control strategies could also be useful. Tasks such as controlling injection velocities, constraining tip positions and aligning injection needle can be automated. Other portions of the tasks, such as cell selection are best left to human intelligence. A series of validation experiments were performed to evaluate the accuracy of microinjection, augmentation, and hybrid strategies with the following three augmentation strategies:

1. Compliant – The robot complies with the scaled user forces.
2. Augmented – Where in addition to compliant motion, asymmetric and non-linear gains and different velocities for different portions of the tasks were used.
3. Supervisory – Where in addition to augmentation, the user selects the point of injection, and the injection is automated.

An embryo was manually selected and captured with the holding pipette, moved to the injection portion of the slide, and brought into focus. The following protocol was then used for the experimental validation.

1. Keep the cell fixed relative to the robot (using the holding pipette),
2. Guide the injecting pipette to the edge of the embryo,
3. Insert to puncture the membrane (using an injection strategy), hold and deposit the material, and
4. Remove the micropipette out of the cell.

The injected embryo is visually inspected for survival. Cell death can be easily detected by changes in the cytoplasm and volume. A surviving embryo is considered successfully injected, while the death of a cell is considered an error. Current literature on microinjection strongly indicates that other than cell damage; purity, quantity, size and nature of DNA injected affects survival of the cell after injection. Thus, to rule out these factors from our initial results, we choose not to inject DNA. We would consider the effects of these factors in the next phase of our experiments.

The operator performed multiple trials to fine-tune the gains for insertion and withdrawal strategies based on subjective evaluation of the ease of use and human factors.

## 3 Materials

The experimental setup (Figure 2) consisted of a Leica DMIL inverted trinocular microscope providing Brightfield, Phase Contrast and Integrated Modulation Contrast optics. With 10X and 40X objectives, and telescoping 10X eyepiece attachments, up to 400X magnification was available. A Narishige mechanical micromanipulator was attached to the microscope. This passive micromanipulator was equipped with an adapter for attaching the holding pipette. The holding pipettes were attached to an oil-filled syringe system driven by a micrometer drive. Standard 0.5-micrometer pre-pulled micropipettes (WPI Instruments, Inc.), and custom pulled (at the Johns Hopkins Transgenic Core Laboratory) holding needles were used for our preliminary experiments.

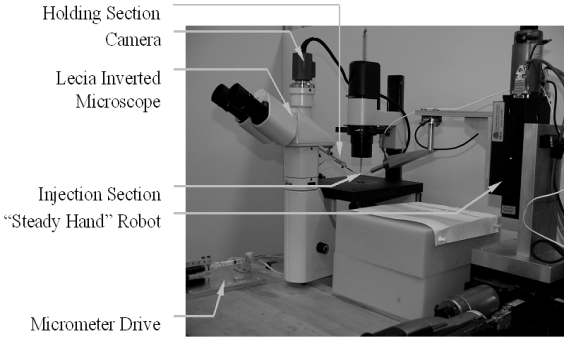


**Fig. 2.** Experimental setup

The "Steady Hand" robot was instrumented with an end-effector (Figure 3) integrating an injecting pipette adapter, a tool tip force sensor and a user force sensor. A CCD camera was attached to the camera port of the trinocular microscope for visual augmentation. The camera was connected to the Matrox™ Meteor II digitizer and also a video recorder for documentation and further analysis. The JHU "Steady Hand" robot and simple force controller used for these experiments is described by Taylor et al in [10]. The custom end-effector is shown in Figure 3.

## 4 Preliminary Experiments

The three different cooperative modes described in section 2 were evaluated with the validation experiments. Only two embryos were injected in compliant mode, and both cells survived after microinjection. Additional trials were performed using augmented and supervisory modes in the interest of time. Eight microinjections were performed for the augmented approach. The path of retraction was restricted to the injection path, and therefore faster velocities could be used for retraction without the fear of additional damage to the cell. This limits the extra time during which the pipette is positioned in the cell, and does not appear to cause any extra damage to the membrane. It also allows more time for the cell membrane to seal itself. The injection path

was chosen to be planar and perpendicular to the cell to avoid cell membrane to avoid damage. Figure 5 shows the user forces and the tool position during one augmented microinjection. Embryos were visually inspected after injection, and survived all eight microinjections performed with the augmented approach. Ease of operation significantly improved with the reduction of the velocities and use of injection and withdrawal strategies.

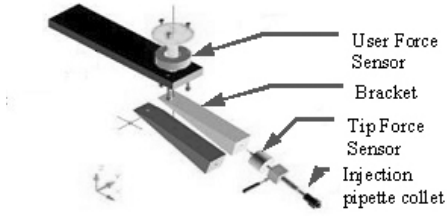


**Fig. 3.** Augmented End-effector for injecting pipette

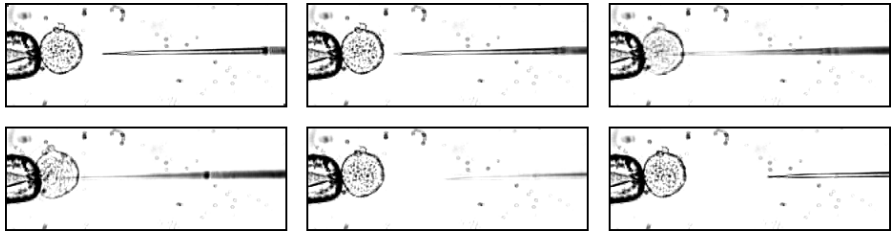

**Fig. 4.** Robotic microinjection of a mouse embryo.

The supervisory approach, allowed the user to position the end-effector in contact with the cell, and the robot then executed a position based injection strategy by moving forward by a fixed distance, holding the tool in the cell, and then retracting back to the injection position. The user then could retract the robot farther away. The supervisory mode was used for 12 microinjections. All embryos were visually inspected, and survived the microinjection.

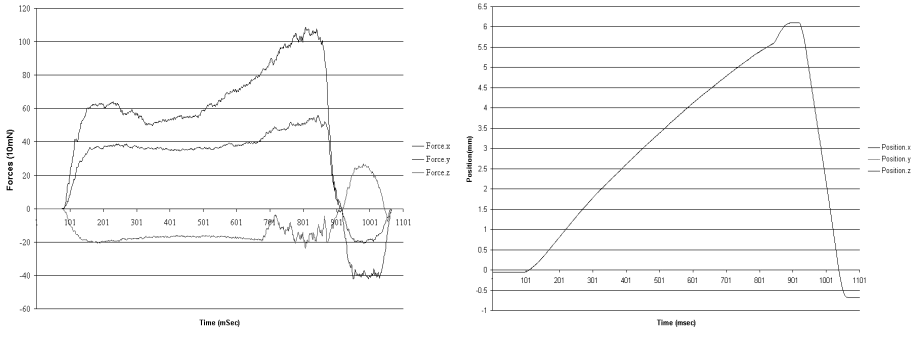


**Fig. 5.** User forces nd tool position for an augmented puncture and pronuclear microinjectionm

We are currently developing vision based methods for automated detection of the embryo and the needle tip. Our aim is to develop suitable virtual fixtures, limiting the workspace and providing navigation to the injection target on the cell. The images from the camera are segmented to detect the egg and the pipette. The pipette direction, and location of the egg are then used to establish suitable direction of motion. Figure 6 shows preliminary results.

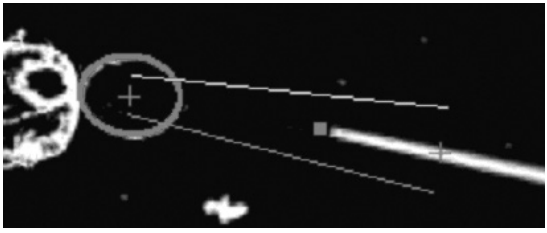

**Fig. 6.** Segmentation of the egg, pipette direction, and a virtual fixture for guiding the user to the cell

**Table 1.** Completion times for different stratergies

| Mode | Time Required for Injection | |
|---|---|---|
| | Average (ms) | Standard Deviation |
| Augmented | 747 | 0.067 |
| Supervisory | 678 | 0.024 |

Table 1 contains average times for performing a microinjection in augmented and supervisory modes. As a passive micromanipulator was used to select and hold the embryo these times are only indicative of the speeds used for microinjection, and are preliminary. Further research is needed to establish total time needed for performing the entire task. Multiple user trials are planned with an improved setup to establish task completion times.

## 5 Conclusions

This paper has reported preliminary experiments for using a cooperative robot system for cell manipulation. These experiments demonstrate efficacy of pronuclear microinjection using a cooperative robot, although further research is needed to refine these results. These experiments resulted in a 100 percent survival rate for all three modes. Although these results are promising, these experiments were limited to exploring the efficacy of microinjection using the cooperative approach. No genetic material was injected. Injection of genetic material may affect the survival rate of the embryos.

These initial experiments indicate a supervisory mode might be best suited for these tasks and appropriate human/machine user interfaces for sensing, and incorporating the user's intention seamlessly will be addressed in our future work. Further research is needed in both force and vision based methods, accounting for difficulties such as collection of cell material on the injecting pipette during microinjection, and presenting the viscosity of the medium in which the cells are contained to the user.

These experiments were designed in collaboration with trained users, but the operator was a graduate student – not trained to perform microinjection on conventional setup. Moreover, these experiments required a time consuming preparation of the embryo before microinjection because a passive manipulator was used for the holding pipette. A redesign of the experimental platform with custom, compact cooperative micromanipulators for both holding and injecting pipettes is currently planned, and will alleviate this difficulty.

Future experiments include an analysis and comparison of performance using different force gains with different trained users and comparisons between conventional setup and our augmented procedures. Currently planned work also aims to extend these results by integrating vision based virtual fixtures in the force control, and replacing the current tool tip force sensor with one of greater resolution. Adding directional constraints and workspace limits improves the ease of operation. It may also significantly improve completion time with the redesigned experimental platform.

**Acknowledgements.** We would like to gratefully acknowledge Ms Mitra Cowan, Director, Transgenic Core Laboratory, Johns Hopkins University School of Medicine and her colleagues for their aiding us in developing an experimental setup. We thank Prof. Gregory Hager for his suggestions on the using computer vision for virtual fixtures. This work was principally supported by a National Science Foundation (NSF) Small Business Innovative Research (SBIR) Grant #0215025, 2002. Additional infrastructure and other support was provided by the NSF CISST ERC under Cooperative Agreement EEC-9731-748, by NSF Grant IIS-98-01684, and by Johns Hopkins internal funds.

## References

[1] B. Hogan, F. Constantini, and E. Lacy, "Manipulating the Mouse Embryo: A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1986.

[2] R. Taylor, P. Jensen, L. Whitcomb, A. Barnes, R. Kumar, D. Stoianovici, P. Gupta, Z. Wang, E. deJuan, and L. Kavoussi, "A Steady-Hand Robotic System for Microsurgical Augmentation", International Journal of Robotics Research, Vol. 18 Issue 12, pp 1201–1210, 1999.

[3] S. Yu, B. Nelson, "Microrobotic Cell injection", Proceedings of ICRA 2001, pp. 620–625, 2001.

[4] A. Codourey, M. Rodriguez, I. Pappas, "A Task-oriented Teleoperation System for Assembly in the Microworld" Proceedings of ICAR'97: Int. Conf. on Advanced Robotics, 1997.

[5] R. Kumar, G. Hager, A. Barnes, P. Jensen, R. H. Taylor, An Augmentation System for Fine Manipulation, Proc. Medical Image Computing and Computer Assisted Intervention, Springer-Verlag, Lecture Notes In Computer Science vol. 1935, pp 956–965, 2000.

[6] I. W. Hunter, L. A. Jones, M. A. Sagar, S. R. Lafontaine, and P. J. Hunter, "Ophthalmic microsurgical robot and associated virtual environment," Computers in Biology and Medicine, (25) 2: 173–182, 1995.

[7] S. E. Salcudean, S. Ku, and G. Bell, "Performance measurement in scaled teleoperation for microsurgery," First joint conference computer vision, virtual realtiy and robotics in medicine and medical robotics and computer-assisted surgery, pp. 789–798, 1997.

[8] C. Ramussen, G. Hager, "Joint Probabilistic Techniques for Tracking Multi-Part Objects" (with C. Rasmussen). IEEE PAMI, 23(6): pp. 560–576, 2001.

[9] A. Bettini, S. Lang, A. Okamura, G. Hager, “Vision assisted control for manipulation using virtual fixtures: experiments at macro and micro scales”, Proc ICRA 2002, pp 3354–3361, 2002.

[10] R. Kumar, P. Berkelmen, P. Gupta, A. Barnes, P. Jensen, L. Whitcomb, and R. H. Taylor. "Preliminary experiments in cooperative human/robot force control for robot assisted microsurgical manipulation", Proc ICRA 2000, pp 610–617, 2000.