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Preliminary Experiments in Robot/Human Cooperative Microinjection

Rajesh Kumar
Foster-Miller, Inc. Waltham, MA
rkumar@foster-miller.com

Ankur Kapoor, Russell H. Taylor
Johns Hopkins University
{kapoor, rht}@cs.jhu.edu

Abstract

This paper reports preliminary experiments with a cooperative robot system to augment single cell manipulation tasks. The JHU "Steady-Hand" robot configuration for cell manipulation is reported. Stable force control laws for the "Steady Hand" are revisited. Preliminary experiments validating stable insertion of a micropipette in a mouse embryo are detailed along with formulation of vision based tracking and augmentation. These preliminary experiments demonstrate promise of cooperative augmentation in single cell manipulation tasks.

1 Introduction

This paper describes the first steps in the development of our Augmented Micromanipulation System (AMS) project[1]. The AMS is being developed to provide a flexible and intuitive means of performing micrometer scale laboratory biomanipulation tasks. Examples of these tasks include manipulation of individual cells, and injection of genetic material into cells. Non-contact manipulation (e.g. laser-trapping) is not appropriate for these tasks, and they are currently performed with the help of joystick driven mechanical micromanipulators and appropriate stabilization, fixation, and visualization. Only visual feedback from the stereo microscope is available to the user. By contrast, 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. This allows intuitive execution of these tasks by taking advantage of the precise manipulation capabilities of a cooperative robot, analytical abilities of a computer, and the intelligence of a human.

This preliminary research used the pronuclear microinjection of mouse embryos as the example task for validation experiments. Transgenic animal models (Figure 1) are a very common method for gene therapeutic and other genetic research. These animals are often created with pronuclear microinjection (Figure 2). The male and female pronuclei are separate and visible for some period of time (several hours for mice) following the entry of the sperm into the oocyte. The *trans*ferred *gene*tic (*transgene*) DNA is introduced into the zygote in this *pronuclear* period immediately following fertilization.

The success of the microinjection technique relies heavily upon the microinjection of the transgene into a relatively large group of accurately timed embryos from a reproductively synchronized group of female embryo donors. The embryo transfer to a suitable recipient female and the construction and preparation of the transgene DNA fragments injected also significantly affect the results.

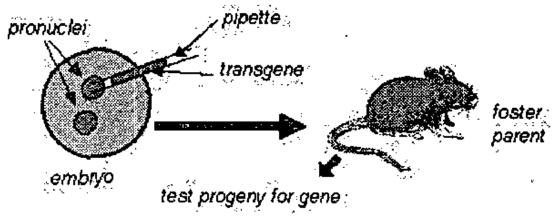

Figure 1: Overview of transgenic mouse model process

A microinjection system for pronuclear microinjection consists of an inverted stereo microscope with Hoffman

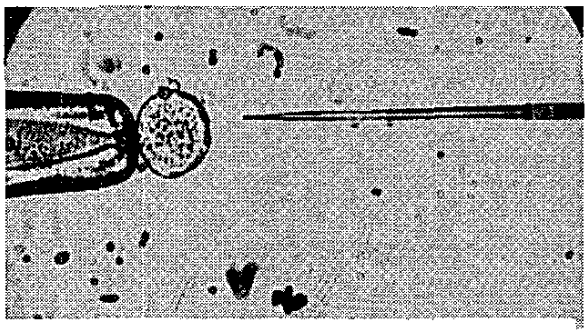

Figure 2: A holding pipette (left) holds the mouse embryo during the injection with the injecting pipette.

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phase contrast or Nomarski optics, configured on a sturdy vibration free base with a holding pipette and an injecting pipette held with mechanical micromanipulators attached to the microscope. The holding pipette holds the cell with light suction while the injecting pipette is used to inject the genetic material. The pipettes are connected to syringes providing required pressure. The holding pipette syringe system is often combined with a micrometer drive for ease of operation.

Prior published work also cites the utility of using a robot for performing this task. Published 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 [1], and Codourey *et al* [2]. Su and Nelson present a custom micromanipulator for teleoperated microinjection. They also use custom fixtures created to hold the cells in place during the process for easier operation. Su and Nelson also report successful autonomous injection into five embryos.

Image based segmentation and visual tracking of cells has been attempted by several groups (e.g. Su and Nelson [1] and Ramussen and Hager [3]). Segmentation of the egg and the holding pipette is relatively easy. However, due to the high magnification used for this task, the injecting needle is completely in focus only just prior to the microinjection and its tip is harder to segment. The direction of the needle can still be reliably estimated.

This paper is organized as follows: Section 2 reviews the JHU "Steady-hand" robot, and its reconfiguration for the micromanipulation tasks. Section 3 reviews simple force control for cooperative manipulation. Section 4 reports the basic methods for segmentation of the images obtained during the process. Section 5 details experimental protocols. Section 6 reports preliminary experimental performance evaluation and Section 7 contains conclusions from this work.

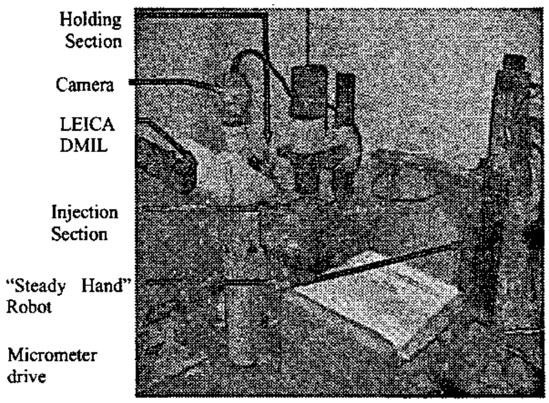

Figure 3: The JHU "Steady-Hand" reconfigured for cell manipulation

2 Experimental Setup

This Section first describes the "Steady-Hand" robot and experimental setup (Figure 3) employed in these experiments, and reviews conventional and experimental protocols.

2.1 JHU "Steady Hand" Robot

The JHU "Steady Hand" robot [4] is a 7-degree-of-freedom manipulator with XYZ translation at the base for coarse positioning, two mechanically constrained center of motion rotational degrees of freedom at the shoulder, and instrument insertion and rotation stages. This robot has overall operational positioning precision under 10 microns, with position encoder resolution of 0.5 micron. The base stages are controllable under 5 microns. The rotational degrees of freedom were not used for these experiments, and the injecting micropipette was attached to the "Steady Hand" base with the aid of the custom adapter shown in Figure 4.

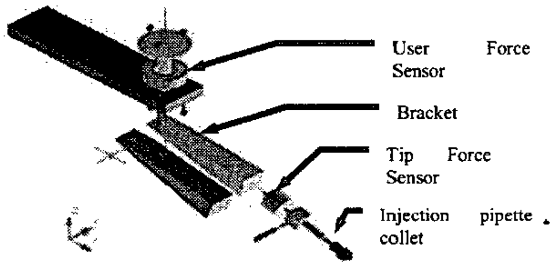

Figure 4: The augmented end-effector

2.2 "Steady Hand" Controller Hardware

An 8-axis DSP series controller card (PCX/DSP, Motion Engineering, Inc., CA) provides servo control using Analog Devices ADSP-2105 40MHz DSPs. An industrial PC computer (Pentium IV, 2.0GHz, 512MB RAM, Windows 2000) houses the motion controller and is used as the application workstation. The PC also contains a 16-bit ATI F/T DAQ card for sensing user forces, and Ni-DAQ Lab-PC+ card with a 12-bit ADC for acquiring forces from the tool tip force sensor.

The robot is programmed in C++ using the JHU modular robot control (MRC) library – a library of C++ classes providing Cartesian level control. It includes classes for kinematics, joint level control, sensor support, peripheral support, and network support. Some exception and error handling is also built in. A variety of I/O devices including serial and parallel ports, ATI force sensors, joysticks, digital buttons and foot pedals are supported.

2.3 Controller Implementation

We used a simple force proportional set point controller detailed on [5], on the "Steady-Hand" robot implemented using the JHU MRC library. This force controller (Figure 5) provides force control with an update rate of 1000 Hz. The image processing component of the controller (supposed to operate at 30Hz), was not used in these experiments.

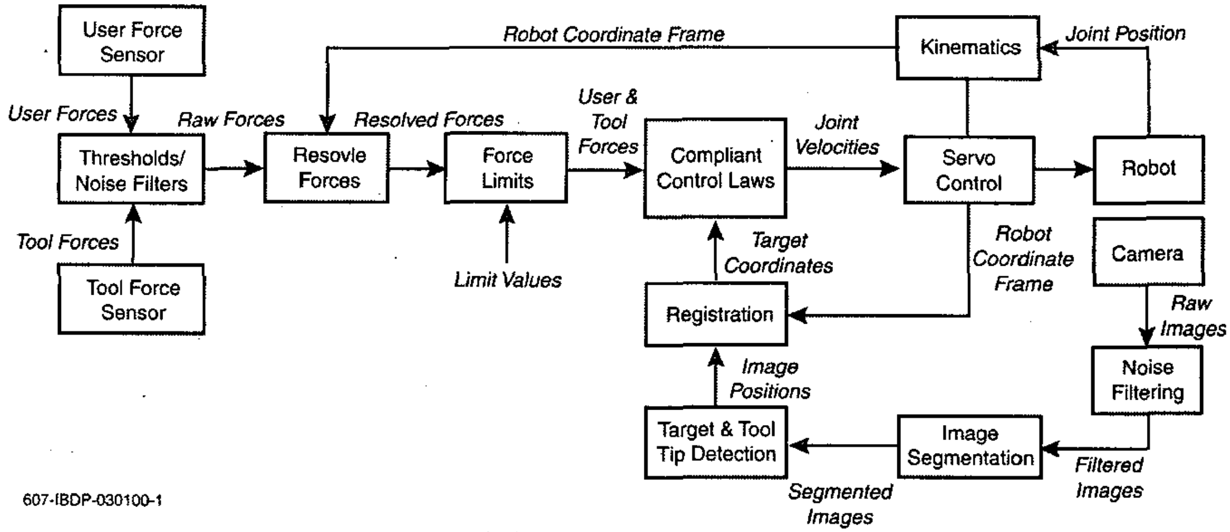

Figure 5: Block Diagram of System Control Law

2.4 Experimental Hardware

In addition to the "Steady Hand" robot, and the tool holder and tool tip force instrumentation (force sensor, amplifiers, analog to digital conversion) the experimental platform (Figure 3) consisted of the following hardware. The Leica DMIL inverted trinocular microscope provided 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. A CCD camera was attached to the camera port of the trinocular microscope for visual augmentation. The camera were acquired by was connected to the Meteor II digitizer and also a video recorder for documentation and further analysis.

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 these preliminary experiments. The experimental workstation was configured on a heavy steel plate on a sturdy vibration damped table.

3 Force Control

This section reviews the basic force control laws (from [5]) used for this research. Nonlinear arm dynamics are highly attenuated in the "Steady Hand" robot especially in translation joints used for this research due to highly geared electric actuators (0.002 m/rev). These joints are individually controlled by a high-gain PID control loop providing a closed loop joint position bandwidth of about 20 Hz. For practical purposes the "Steady hand" robot can be approximated by a position-controlled device.

For a position controlled mechanism with joint position $x(t)$ and joint velocity $x'(t)$, the tool-tip force at equilibrium is $f(t) = k_t x(t)$. If we neglect any tracking errors, then $x(t) = x_d(t)$and $x'(t) = x'_d(t)$ where $x_d(t)$ or $x'_d(t)$ is the control input. For a constant desired tool-tip force, f_d the control task is to ensure that the force tracking error, $\Delta f(t) = f(t) - f_d$, converges asymptotically to zero, i.e. $lim\ \Delta f(t) = 0$. Assuming that tool-tip force, $f(t)$, and the plant state, $x(t)$ and $x'(t)$ are measured, the control law $x_d(t) = k_f \int \Delta f(t)\, dt$ gives stable first order closed loop force dynamics of $\Delta f'(t) = k_f k_t \Delta f(t)$.

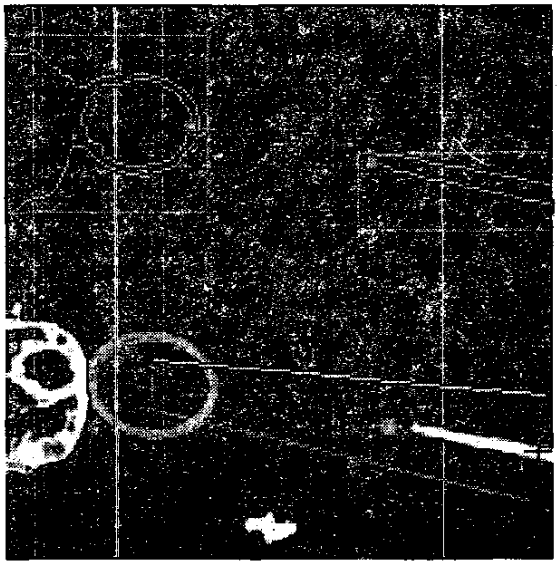

Figure 6: Segmented Connected Components (top), and contours for the embryo, the pipette tip, and virtual fixture for guiding the user to the cell (bottom).

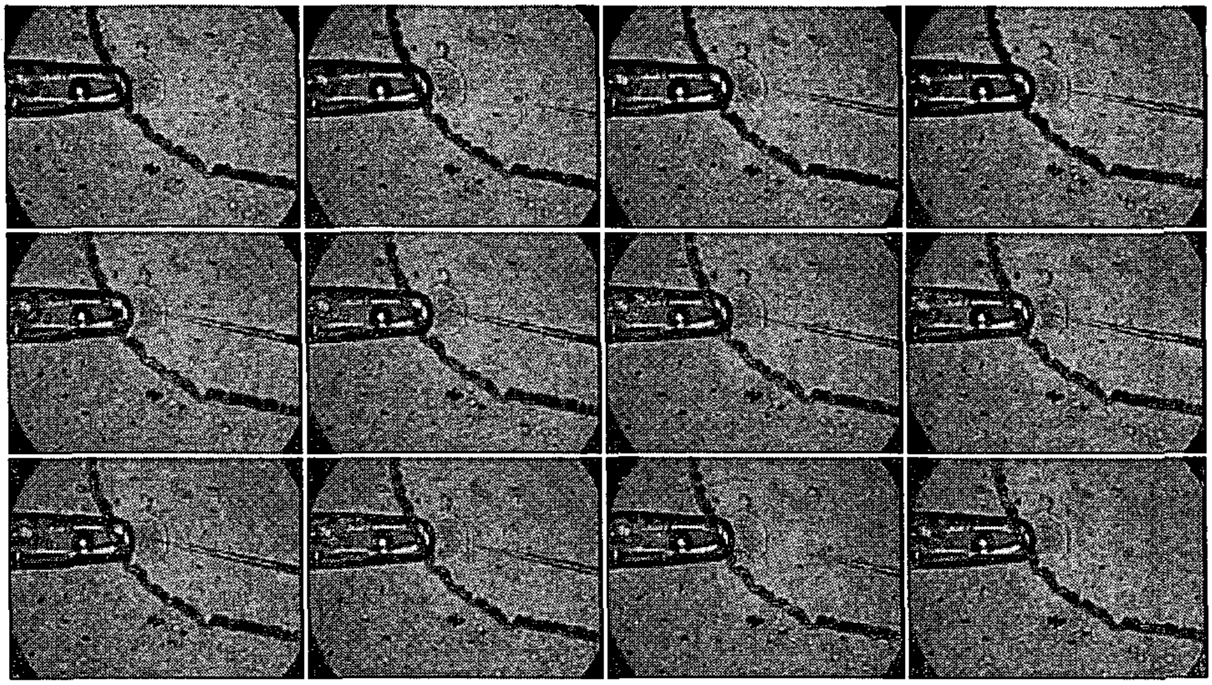

Figure 7: Compliant microinjection of a mouse embryo.

The desired force is computed in real-time by scaling down $f_{tool}(t)$ the force exerted by the user on the force-instrumented tool handle, such that $f_d(t) = \alpha\ f_{tool}(t)$ where α is the scale factor of the tip force to handle force. For the "Steady Hand" robot a wide range of scale factors has been experimentally tested.

4 Automatic Target and Tool tip Direction

This section outlines the image processing for detecting the target (embryo) and the injecting pipette direction for constructing virtual fixtures to guide the user to the target.

A manually defined sub-image in the image obtained from the digitizer is processed to extract connected components corresponding to holding pipette, the embryo and the injecting pipette. This includes noise filtering, inversion, and thresholding. Morphological opening and closing steps are applied to smooth the contours in the threshold images.

The boundaries of the two connected components thus obtained are then processed to detect the center of the egg, its boundary, and bounding box, as well as the injecting pipette direction and tentative injecting pipette tip. Since the injecting pipette is only in view just prior to the injection, only the stable pipette direction is used for constructing a virtual fixture guiding the user to the embryo.

5 Experimental Methods

This section reviews experimental protocols used for the pronuclear microinjection. The pronuclear microinjection task was observed as performed by trained users, and conventional operations were analyzed.

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.

a) Keep the cell fixed relative to the robot (using the holding pipette),
b) Guide the injecting pipette to the edge of the embryo,
c) Insert to puncture the membrane (using an injection strategy), hold and deposit the material, and
d) Remove the micropipette out of the cell while constraining it to move back only along the direction of insertion.

The injected embryo is visually inspected for survival. A surviving embryo is successfully injected, while the death of a cell is considered an error.

Three different cooperative modes were evaluated with the validation experiments - compliant, augmented, and supervisory.

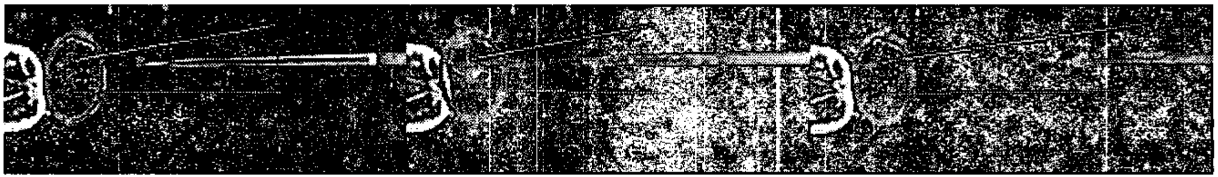

Figure 8: Visual tracking of a microinjection. Stored data was used to construct this segmentation of the embryo, injecting pipette and the virtual fixture for assisting the user in navigating to the embryo

6 Experiments

This section reports preliminary experiments. The task outline above suggests visual augmentation by visual servoing to align the micropipette, an automatic puncture strategy and virtual fixtures to constrain the path of insertion and removal. The task outline also suggest that portions of the task could be automated, for example aligning the pipette, and puncture of the cell membrane. Other portions of the tasks, such as selection of a particular embryo, positioning inside the pronucleus, and holding time required to deposit the right amount of genetic material especially in simple syringe type microinjection setups is best left to human intelligence. The task outline also suggests that different position, velocity and force control strategies could be useful. These could be integrated with strategies based on visual feedback such as motion to target, constrained motion in image coordinates, and alignment with respect to features detected in the images.

The resolution of the tool tip sensor was found to be inadequate for sensing forces other than the puncture of the cell membrane. The forces experienced during the puncture event, and were observed to be consistently within 5-10mN. A further experiment was performed to measure contact and manipulation forces. A holding pipette was mounted on the "Steady Hand" robot instead of the sharp injecting pipette and the embryo was manipulated with the two holding pipettes, including squeezing and pushing at the embryo. The magnitude of the contact and manipulation forces was observed to be in the 10mN range. Future research will include a more sensitive force sensor to be able to better use the tool tip force information.

Three different modes were used for the validation experiments. In the complaint mode (Figure 7), the user manually injected the embryo with no augmentation. Only 2 embryos were injected in this mode, and both cells survived after microinjection. Further microinjections in the compliant mode were omitted in the interest of time, and saving the embryos for more interesting modes. Figure 7 shows one of the microinjections (with a non trained user – the graduate student performing these experiments).

6.1 Augmented mode based microinjection

In order to inject into the cell with minimal damage, the needle must withdrawn as quickly as possible. To facilitate this, the augmented mode used different velocities for injection and withdrawal, that is, for the same user force the velocities away from the cell was a constant times the velocities towards the cell. This limits the extra time during which the needle 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 user also had the option of locking one or more axes of the robot on click of a button by setting the force scaling gains zero. Typically, this was used to restrain the needle in a plane once it was in focus. Eight microinjections were performed for the augmented approach. The injection path was chosen to be planar and perpendicular to the cell to avoid cell membrane damage. 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.

6.2 Supervisory mode based microinjection

In the supervisory approach, the user positions the injection needle close to the cell, with the robot velocities proportional to the applied user force. Once the user is satisfied with the positioning of the needle, the robot is commanded to 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. As with augmented mode the injection path is perpendicular to the cell. 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.

6.3 Vision based navigation

Since the embryo was manually collected and located under the microscope, vision guidance was not integrated into the control for these experiments. However, the vision components of the system were independently tested. The images collected from the experiments were post-processed to detect the embryo, the tip and direction of the needle. Our virtual fixture was a manually defined band of constant dimensions around the line joining the cell and center of needle, with a taper towards the cell. Once enabled, the motion of the needle was restricted to this region. Figure 8 shows the segmented egg, the needle tip and direction and the virtual fixture prior to, during and after microinjection.

7 Conclusion

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. However, these experiments were limited to exploring the efficacy of microinjection using the cooperative approach. No DNA was injected.

Microinjection was possible even with the basic force scaling compliant mode. Two other modes – augmented and supervisory, were also explored. Ease of operation was significantly improved with directional constraints and workspace limits in these two modes. The supervisory mode was the easiest to operate. It is possible that the puncturing task is best performed automatically, in a supervisory mode. This would facilitate more uniform microinjection of embryos and adjust for effects such as accumulation of cell material on the pipette that are inherent in a batch mode paradigm used in microinjection tasks.

These initial experiments indicate a supervisory mode might be best suited for these tasks. Therefore, appropriate human/machine user interfaces for sensing, and incorporating the user's intention seamlessly will be address 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. Future experiments including a comprehensive subjective evaluation will be performed with trained users.

Currently planned work to extend these results includes integrating the vision based virtual fixtures in the force control, and replacing the current force sensor with one of greater resolution. A redesign of the experimental platform with custom, compact cooperative micromanipulators for both holding and injecting pipettes is also planned.

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