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New Developments Towards Automated Blastocyst Microinjections

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***Abstract*—This paper presents results related to our latest semi-automated blastocyst microinjection system. Here, the improvements made to the microinjection system are described and evaluated. First, after replacing the original piezo-electric kinematic stage by a DC motor-based robot manipulator, experimentation showed that the speed and the precise motion control of pipettes were improved. Second, by introducing an X-Y stage into the system, to manipulate the Petri dish around the microscope's field of view, multiple microinjection speed was improved. Third, by using SSD template matching to track the injection pipette, rather than the cross-correlation template matching algorithm used in the original system, improvements were made to pipette localization. Under human control, this new semi-automated system gives improved microinjection performance metrics compared to previously obtained results. The system is also providing implicit human knowledge of the microinjection process via the human-control interface. It is the encoding of this knowledge that will lead to the first fully automated system. The semi-automated microinjection system is being tested and evaluated in the AMC at UNC-Chapel Hill.**

Index Terms – Cell injection, biomanipulation, teleoperation, microrobotic system.

I. INTRODUCTION

Currently, research in genetics and associated biomedical areas rely a great deal on the use of genetically modified mice for the discovery of gene functions and for the understanding of how defect in genes lead to the development of diseases [1]. Gene-targeted mice, for example, are often used as models of a wide range of serious human afflictions, including diabetes, arteriosclerosis, hypertension, Alzheimer's disease, and cancer [2].

Gene-targeted mice, also known as knocked-out mice, are commonly created by the injection of genetically altered embryonic stem (ES) cells into early embryos during the blastocyst stage. These operations are called blastocyst microinjections. The success of these microinjections depends, to a large extent, on meticulous manipulations of the delicate cells. Therefore, the skills of the person performing the operations have a significant impact on the results of the microinjections.

According to published literature [3], operators need up to one full year of training to become proficient at injecting blastocysts. However, even with all that training, low survival rates of microinjected cells are encountered, often being between 40% and 70% [4].

The problems that affect the efficiency of blastocyst microinjections, as well as other biological micromanipulation tasks, are related to human errors and to the lack of repeatability. Thus, one possible way to address these problems is to automate the manipulations. A fully automated microinjection system is the goal of our research.

A pioneer in the area of embryo biomanipulation automation was Ogawa. Together with Takahashi, Mizuno, Kashiwazaki, Yamane and Narishige, Ogawa put together a computer-controlled system for the manipulation of eggs and early embryos in 1985 [5]. Their system depended heavily on operator inputs, and the motion control of the manipulator worked in open loop based on manually defined starting and ending positions. Nevertheless, the system proved to successfully accomplish tasks such as bisection and microinjection. Ogawa and fellow researchers continued to work on automation improvements, and in 1992 they reported on a new system that automated the subzonal insemination of mouse ova [6]. In this case they used computer vision techniques (i.e. template matching) to locate the holding and injecting pipettes at the beginning of each microinjection procedure, and from that point the microinjection operation was performed in open-loop.

Within the past two decades other researchers have also worked in areas related to biomanipulation automation, studying and proposing solutions to problems such as the positioning [7], [8], [9], holding [10], [11] and injection [12], [13] of biological cells. By the beginning of the 21st century, research on the automatic visual tracking of cells started to be reported upon [14], [15], and in 2002 Sun and Nelson introduced visual servoing as a major technique to enable closed-loop control in an automated cell injection system [16]. Their paper, which has become a classical reference in the biomanipulation automation area, describes an automated system for embryo pronuclei DNA injection based on a single general-purpose microrobot and on a hybrid visual servoing scheme. On that same year, Zhao *et al.* [17] also reported on a similar system using two micromanipulators which, following user inputs, could also autonomously capture the target cells for injection.

Recent research published in this area has demonstrated an apparent step back from the full automation concept and a general move towards the development of augmented reality systems to aid and improve manually controlled microinjections [18], [19], [20]. Many recent studies have also been concerned with a better characterization of the

cell membranes and of the forces involved in the microinjections [3], [21], [22]. This phenomenon may be due to the fact that, as Arai put it, "micromanipulation tasks are versatile. So it is difficult to realize a full automation system" [23]. Researchers may be following Arai's suggestion to start by classifying the basic operations. However, there is an ever-growing need for improved consistency and efficiency of biomanipulation operations which can only be satisfied by full process automation. Therefore, we continue the push towards a fully automated system even without the knowledge of all of the intricate details of cell microinjections.

The idea is to let the system learn the task from skilled users, who have vast but implicit knowledge about it. Therefore, we have created a framework that allows a computer to observe and register all human actions during microinjection experiments.

The developed framework consists of a semi-automated blastocyst microinjection system that is teleoperated via a graphical user interface running on a desktop computer. This configuration enables the construction of a knowledge database from which intelligent controllers will be developed to fully automate the microinjection process.

This paper presents the latest improvements to the developed semi-automated system, which is described in greater details in [24], and which is currently in use at the Animal Models Core (AMC) Facility at UNC-Chapel Hill. Here we introduce the use of a new robot manipulator, and present experimental results that show how this new manipulator improves microinjection performance. In addition, an XY stage was incorporated to speedup multiple microinjection tasks. This is also described here, along with improvements and evaluation results of the employed vision system. Finally, preliminary results of blastocyst microinjections are provided.

II. The Semi-Automated Microinjection System

The developed teleoperated blastocyst microinjection system is shown in Fig. 1. The figure shows that the microinjections are performed under the microscope, which provides the necessary optical magnification and illumination levels for proper imaging the injection area. Video of that area is acquired by a CCD camera, and is sent to the desktop computer for displaying and processing. Real-time analysis of the video images determines the exact position of the blastocysts and pipettes during the microinjections experiments, which are recorded as experimental data and which will be later used as feedback information for automated operations.

When using this system to perform the microinjections, the operator sits in front of the computer screen and controls the entire procedure using a joystick. The generated motion commands are recorded as experimental data, and then processed and sent to the motion devices. Using the joystick the user can control: (a) 3-dimensional motions of the injection pipette through the motorized micromanipulator; (b) the activation of the piezo injector; (c) fluid motion on the holding and injection pipettes through motorized micrometer syringes; and (d) the position of the petri dish through the motorized XY stage.

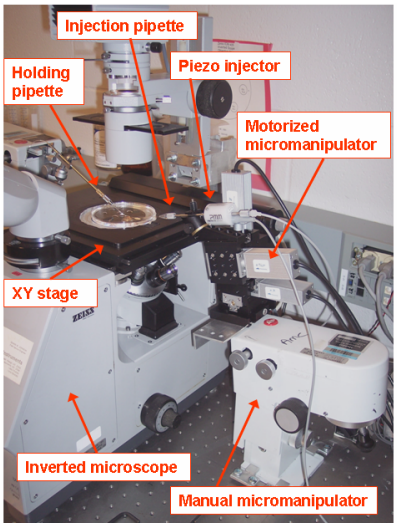


Fig. 1. Microscope stage setup for teleoperated blastocyst microinjections.

The developed computer interface is a key system element for making teleoperated micromanipulations possible. It provides the user with a microinjection environment that resembles a computer game, and allows the operations to be performed from a more comfortable and ergonomic setting; one that imposes less strain to the user's eyes and body. The developed interface also allows the user to monitor and tune the motion devices and the video processing algorithm, what facilitates adjustments for peak performance and for the collection of reliable experimental data.

Initial tests with the system described in [24] showed that it required a few updates to become useful. The main problem lay with the original robot manipulator used, a piezo-electric kinematic stage, which caused excessive vibrations while in motion and tended to damage the embryos during injection. Another problem was the lack of an XY stage, which is essential for multiple microinjection tasks if we want to avoid having the operator go to the microscope stage before each microinjection.

We have addressed the problems mentioned above with the acquisition of an XY stage and a new robot micromanipulator, which were tested and evaluated as described in the next section. The image processing algorithm was also updated from [24] to improve its localization performance and robustness. The description and evaluation of the algorithm updates are presented ahead, followed by a description of preliminary blastocyst microinjection experiments.

III. System Improvements

A. *New Robot Manipulator*

The updated blastocyst microinjection system is based on a new micromanipulator robot that provides vibration-free motions and is faster than the previously employed piezo-electric kinematic stage. That stage, the NewFocus 8082, was found unfit to the task of injecting blastocysts for the following reasons:

i. Excessive vibration during motion: Although not visible by eye, vibrations caused by the piezo-electric motors create fluid motions that make the collection of ES cells for injection impossible. Furthermore, injection experiments showed a high risk of fatal damage to the blastocysts when the NewFocus stage was used (7 out of 12 blastocyst injections were considered a failure by a microinjection specialist because of damage inflicted on the embryos' membrane by pipette vibrations).

ii. Slow motions: The speed of motion of the NewFocus stage was a problem for this application. Its maximum velocity of 1.2 mm/min (or 20 µm/s) was found to be too slow for blastocyst manipulation. Blastocysts typically measure 100 µm in diameter, and operators like fast motions to quickly grab and position them for injection.

iii. Limited lifetime: The NewFocus 8082 kinematic stage is not designed for constant motions since its target application is the alignment of optical devices. The manufacturer specifies a lifetime of 15,000 cycles for each picomotor actuator, and defines a cycle as 1mm of travel range out and back pushing a 5-lb axial load. Therefore, this stage is not appropriate for the blastocyst microinjection system, which needs a robot that can sustain constant motions.

The new Siskiyou MX7600R motorized micromanipulator provides motions that are virtually vibration-free because it is based on DC motors instead of piezo-electric or stepper motors. Successful ES cells collection experiments using this robot confirmed that its motions are smooth enough for the intended application.

The new robot is also able to move at speeds up to 1.7 mm/s of linear velocity while still maintaining its accuracy thanks to built-in encoders. The minimum controllable displacement of this Siskiyou robot is 0.1 µm. This is a much larger value than the 30ηm achieved by the NewFocus stage, but is enough for the blastocyst micromanipulation tasks since the smallest cells involved in the process (the ES cells) typically measure 10 µm in diameter.

In the developed system, the fast displacements and the precise motions required for blastocyst manipulations are achieved by applying an exponential function to the analog commands generated by the joystick, as shown in Fig. 2.

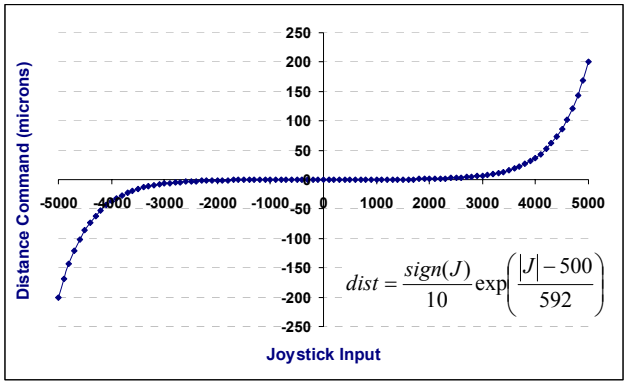


Fig. 2. Exponential function applied to the joystick commands. Modifying the linear joystick values allows for fast displacement and for small and precise motions without changing any of the system's settings.

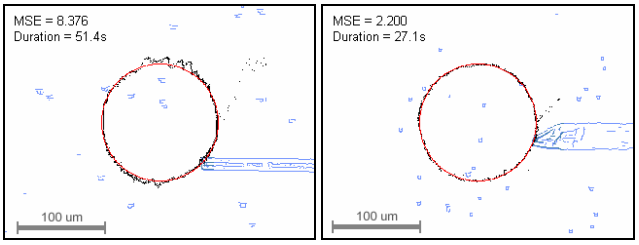
Fig. 3. Joystick function assignments: (1) translate the injection pipette along the X-, Y-, Z- and T-axis, (2) translate the XY stage along its X- and Y-axis, and (3) control the piezo-injector and micrometer syringes.

This way the operator can command small or large displacements without changing any of the system settings.

Furthermore, the joystick's slider bar was set as a velocity gain control to allow for even faster motions, so the operator can easily increase the velocities directly from the joystick. Fig. 3 shows the restructured joystick function assignments for control of the microinjection system.

As a performance comparison between the two robots (and also to evaluate the visual servoing system), experiments were conducted to check how well the system could control the injection pipette motions. The experiments consisted of commanding the robots to follow a circular path with 130 µm in diameter. For both robots, the same visual servoing scheme was used to control the motions, but the control gains were adjusted differently to accommodate for the mechanical differences between them. The obtained results are presented in Fig. 4, which shows time-lapsed pictures of the experiments. The paths followed by the robots are marked with black dots, which represent the position of the injection pipette's tip at each processed video frame. The figures also show the duration of one full turn around the circular path and the mean square errors (MSE) computed from the deviations from the desired path.

The results presented in Fig. 4 show that the Siskiyou micromanipulator was able to complete the task almost twice as fast and with almost a quarter of the MSE achieved by the NewFocus kinematic stage. However, this was mainly due to different calibrations of the visual servoing system, which is based on a PID control strategy. The path-



(a) (b)

Fig. 4. Visual servoing results using (a) NewFocus 8082, and (b) Siskiyou MX7600. The red circle represents the desired path.

following MSE results obtained with the NewFocus stage could have been better if its speed was reduced, but this was not desired since the stage is already too slow for this application. Consequently, the PID values were set to make the stage move as fast as possible, giving rise to the observed overshoots and errors during the path-following experiment.

A measure of the improvement provided by the employed visual control system was obtained by performing the same experiments under operator control. In this case, path following was tested for three conditions:

i. The operator used the joystick to generate motion commands for the NewFocus robot.

ii. The operator used the joystick to generate motion commands for the Siskiyou robot.

iii. The operator used the manual micromanipulator to directly control the injection pipette motion.

The best results from a series of 10 trials for each experiment are shown in Fig. 5. The results show that the MSE obtained with the Siskiyou robot was about half of the value obtained with the NewFocus stage, therefore indicating that the operator had better control of the injection pipette's position when using the Siskiyou robot. On the other hand, the direct manual control of the pipette proved to be faster and more precise than using either of the two robots. The observation is that the operator is very well trained in manual control of the motions of the pipette, but not familiar with the use of the joystick as yet. Therefore, we expect faster and more precise motions as the operator becomes more familiar with the teleoperated system. In any case, when comparing the results in Fig. 5 with the ones in Fig. 4, it is clear that the visual servoing system provides a much finer control over the injection pipette motions. This further motivates the goal of fully automating of the blastocyst microinjection process since better motion control translates into reduced chances of inflicting lethal damage to the embryos.

The last experiment performed evaluated the Siskiyou micromanipulator in an "open-loop" trial using the same path-following experiment. In this case the goal was to evaluate the position control provided by the robot controller unit (the Siskiyou MC2000), which is based on readings from the motor's encoders. This experiment was labelled as open-loop because the visual servoing system was turned off. Here, only the initial pipette location was obtained using the template matching algorithm. All subsequent motion commands consisted of relative motions solely based on expected pipette locations.

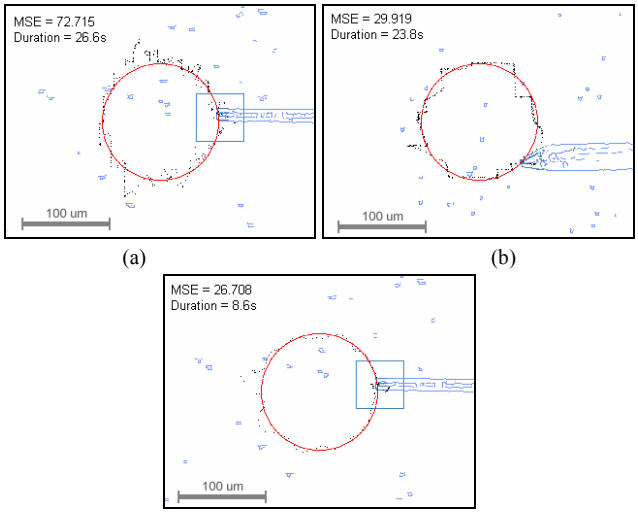


Fig. 5. Manual control of the injection pipette using (a) NewFocus 8082, (b) Siskiyou MX7600, and (c) mechanical micromanipulator.

The obtained results from 10 trials of this last experiment were all very similar. As an example, a time-lapsed picture of one of the trials is presented in Fig. 6. It shows that the open-loop path-following trial resulted in high MSE. In contrast, a qualitative analysis of the path followed shows that it was reasonably good. This is important because it shows that position information obtained from the robot can be used to improve the injection pipette's visual tracking algorithm. In the developed system this position information is being used to adjust the location of the pipette's search window.

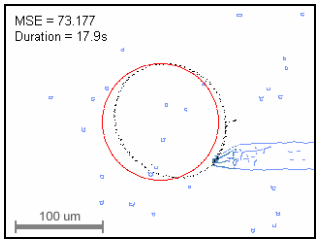


Fig. 6. Open-loop servoing using the Siskiyou MX7600 robot.

B. XY Stage

The new and improved semi-automated blastocyst microinjection system incorporates an XY stage to move the petri dish around during the procedures. This is useful because it facilitates the collection of ES cells and because it allows the operator to remotely carry out multiple microinjections without the need to physically go to the microscope stage to move the petri dish.

With the XY stage, the operator only goes to the microscope stage to setup the working wells at the beginning of the microinjections. After that all operations are performed from the computer station using the joystick. This saves time when multiple microinjections are performed, especially because ES cells and blastocysts are typically placed at different locations within the same working well. Furthermore, several working wells may be put on the same petri dish, so there is also the need to be able to move each of those sites to the microscope's field of view. An example of a typical petri dish setup is shown in Fig. 7.

The installed XY stage is directly controlled from the system's joystick or, alternatively, from buttons placed on the graphical user interface. It presents a step resolution of 10 µm in each direction, and can move at speeds up to 45 mm/s. A custom controller board drives this stage, and also provides storage area for the coordinates of 10 locations, allowing for prompt motion between different sites. This

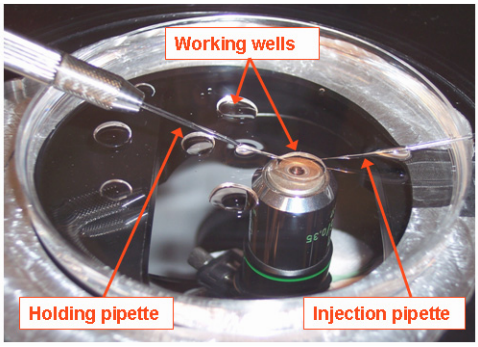


Fig. 7. Typical Petri dish setup. Each working well contains both blastocysts and ES cells for injection, and several working wells may be put on the same petri dish.

feature helps to further speedup multiple microinjection tasks by enabling precise and quick motions between the blastocyst and the ES cells sites.

C. *Improved Vision System*

On our previously reported microinjection system [24] the employed vision processing was introduced as consisting of pipette tracking algorithms based on cross-correlation template matching, and a blastocyst localization algorithm based on Hough transforms. These were (and continue to be) used as source of information to record user actions and their respective reactions during the microinjection experiments. Another major aim of the vision system is to locate those objects in real-time, what enables the application of visual servoing techniques on a future fully automated system.

The previously reported evaluation results (see [24] and [25]) have demonstrated good localization performance for all three objects of interest: 90% success rate for the blastocyst localizations; 99.8% success rate for the pipettes localizations; and 94% success rate for the automatic selection of the injection area on the blastocysts' trophoblasts. However, these results were obtained either from pre-recorded microinjection videos or from simulated images. Therefore, they reflect expected values and not true performance measurements.

A better vision algorithm evaluation was performed once the system was moved to the AMC's facility. This time, images of blastocysts and pipettes collected with our own system's camera were used for the experiments. The results are presented in Table 1, and reveal even better performances than previously reported. The exception was the success rate obtained for the injection pipette localizations using the original cross-correlation template matching algorithm. This rate was found to be lower than expected due to the fact that the injection pipette tip is very small and does not present many distinctive features. Consequently, the acquired template presented few pixels and poor features, causing the cross-correlation template matching algorithm to fail more frequently than it was expected from simulations. Improvement was obtained by changing the search algorithm to a Sum-of-Squared-Differences (SSD) template matching, as described in [16]. In this case all template pixels are used for matching, so more robust localization results are obtained.

Further improvement to the injection pipette tracking algorithm was obtained by using the position information provided by the micromanipulation robot to adjust the position of the pipette's search window. This provided an error-filtering action that prevents the template to drift off from the real pipette location in noise conditions; however, further experimentation is necessary to obtain a quantitative measure of its effectiveness.

A picture demonstrating the performance of the current vision system is presented in Fig. 8. It shows good algorithm performance even in the presence of occlusions and when edges of different objects merge.

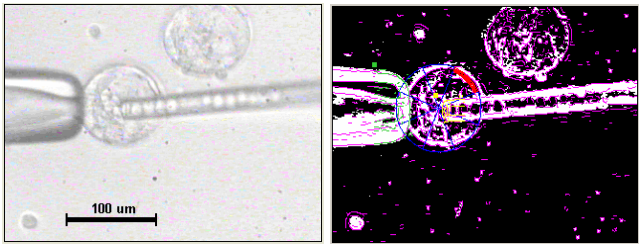


Fig. 8. Original and processed video frames showing good results of the localization algorithms for a common microinjection scene.

IV. Preliminary Microinjection Results

As mentioned earlier, we have performed a few preliminary blastocyst microinjection experiments using the current system setup. These experiments were all carried out by a microinjection specialist, who tested the initial system when it was based on the NewFocus stage, and also the updated system based on the Siskiyou robot. The obtained results, which are presented in Table 2, demonstrate the large performance improvement provided by the new robot micromanipulator. Furthermore, despite the fact that these were just initial experiments, the obtained 81% blastocyst survival rate has surpassed the 40-70% range commonly found for manual microinjections [4].

TABLE 1: Vision System Evaluation Results.

Object	Localization (% correct)	Sector selection (% correct)	Number of images
Blastocyst	98.3%	99.0%	790
Injection pipette	94.6% (cross-correlation)	–	893
Injection pipette	99.3% (SSD)	–	893
Holding pipette	100% (cross-correlation)	–	300

TABLE 2: Preliminary Microinjection Results.

Robot employed	# blastocysts that survived microinjection	# injected blastocysts that survived for 24 hours	Number of injected blastocysts
NewFocus 8082	5	2	12
Siskiyou MX7600R	13	13	16

V. Conclusions and Future Work

This paper presents the latest improvements and updates incorporated into our semi-automated blastocyst microinjection system, including the replacement of the original piezo-electric-based kinematic stage by a new DC motor-based micromanipulator. This updated system was evaluated in this paper by the undertaking of a comparative study that measured the performance of this new micromanipulator robot for a blastocyst manipulation task. The results obtained demonstrated that transferred vibrations were reduced, manipulation speed was increased, and precision motion control was improved. Moreover, in early experimentation, teleoperated microinjections using the new robot have resulted in an 81% blastocyst survival rate, surpassing the 40-70% range typically found for manual microinjections. Therefore, even greater survival rates are expected once the operator becomes more familiar with the controls.

The incorporation of an XY stage to speedup multiple microinjection tasks has also justified its inclusion into the system. Lastly, the results from the vision processing algorithm were evaluated. The results showed a success rate of nearly 100% for localizing objects of interest. This means that the system performs reliable experimental data collection and provides reliable position feedback for visual servoing.

For the near future, regular microinjection experiments are planned, including the implantation of the injected blastocysts into surrogate mothers to confirm they can develop into chimeras. Furthermore, we plan to add artificial intelligence to this microinjection system to fully automate the process. Such intelligence will come in the form of a knowledge-based controller, which will be developed from the knowledge being gathered by the use of the current semi-automated system.

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