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A Micromanipulation System for Automatic Batch Microinjection

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I. INTRODUCTION

The microinjection is a common technique in genetic engineering for transferring genetic material into a cell. It is normally performed on a micromanipulation system that usually consists of an inverted microscope, a micromanipulator, a micropipette and an injector. Manual microinjection is a conventional and widespread practice. However, this approach suffers from low success rate, poor efficiency, and high probability of contamination. In a manual microinjection process, the skill and experience of the operator play a crucial role in achieving a successful injection. It usually takes several months of training and practice for an operator to become proficient in performing such a task. However, even for an experienced operator, the success rate of such manual microinjection may still be very low. This is mainly due to the fact that to execute various steps in a manual microinjection requires fine control of both position and force, which is difficult for a human operator to accomplish consistently. Finally, intervention of a human operator throughout the microinjection process also increases the chance of contaminating the biological organisms. In order to resolve above problems, it is necessary to automate the microinjection process.

Very few studies on the research and development of micromanipulation systems for automatic batch microinjection have been reported in the literature, and no commercial system has been demonstrated to operate automatically for microinjection [1]–[7].

We have developed a micromanipulation system for automatic batch microinjection by automating the key process of penetration in a microinjection.The microinjection system automatically identifies and penetrates each embryo/cell with a constant and fast speed, and in doing so, eliminates the possibility of contamination caused by manual operations. The effectiveness of this force-controlled system has been experimentally demonstrated in the tasks of automatic identification and penetration zebrafish embryos, which are widely used as a model for studying vertebrate development and genetics [8]–[9]. The average penetration force and penetration time for processing an embryo were around 1 mN and 15 seconds, respectively.

II. DESIGN AND IMPLEMENTATION

To achieve batch microinjection of zebrafish embryos, the micromanipulation system is designed to handle multiple embryos without holding them separately. This is achieved by using parallel V-grooves (made on the gel in a petri dish) to array and hold the embryos in multiple rows. The petri dish is mounted on a tilted holder, as illustrated in Figure 1.

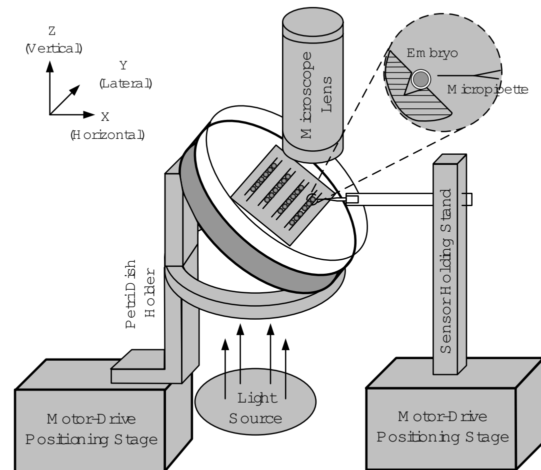

Fig. 1. Schematic illustration of the batch microinjection system.

The holder is mounted on a high-precision motion stage, which can be maneuvered so that at the start of the automatic microinjection process the left end of the top row of the arrayed embryos can be focused by the microscope. Another positioning stage is used to move the micropipette. When the tip of the micropipette and the viewable embryos appear together in the local view of the microscope, their images are captured by a CCD camera. The normalized two-dimensional cross-correlation algorithm is used to identify the yolk of an embryo. Then the number of embryos is determined and the centerline of each embryo and the centerline of the micropipette are marked.

The micropipette is moved laterally to align itself with the centerline of the first embryo in a row, at which point penetration starts with the micropipette moving horizontally towards and then into the embryo at constant speed. A piezoresistive micro-force sensor measures the penetration force, which is then used by a controller using dynamic feedback of the penetration force to decide whether the chorion layer is penetrated, the extent of penetration, and when to stop the micropipette.

In the application of the method of position control with force feedback, the position of the micropipette and penetration force are sampled in two different real-time processes, with the force sampled at a much higher frequency. The first-order and second-order derivatives of the penetration force are

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computed in real-time. When their values meet the requirements, the position control will command the micropipette to stop. The stopping position should be inside the embryo, slightly further inward from the point of penetration.

Upon successful penetration and injection, the micropipette retracts and moves to the next embryo and performs the penetration and injection again. This procedure is repeated the entire batch of embryos has been processed.

III. Experiment Setup and Results

To demonstrate the effectiveness of the proposed approach for batch microinjection with force feedback, an experiment involving the penetration of a group of zebrafish embryos using the prototype automatic micromanipulation system was designed and conducted.

The micromanipulation system consists of a petri dish with a custom-design holder, a modified piezoresistive force sensor bonded to a micropipette, two sets of high-precision motion stages, an imaging unit and a dynamic strain-meter. All the instruments were mounted on a vibration isolation table. Figure 2 shows an overall view of this system.

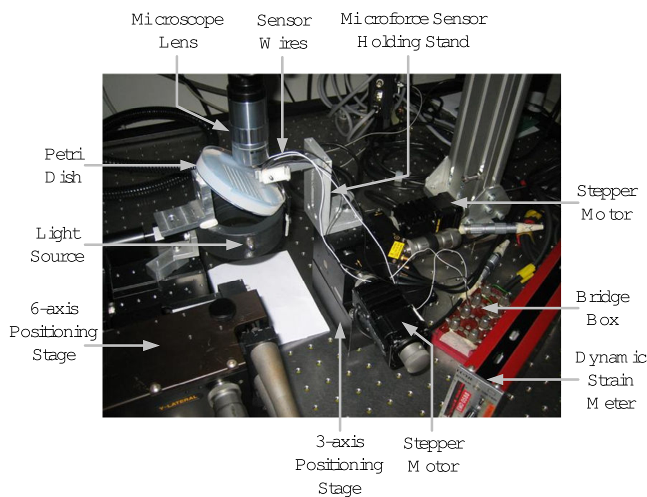

Fig. 2. Setup of the micromanipulation system for batch microinjection.

This batch microinjection experiment involved three zebrafish embryos, which is within the range of the limited view of the lens of the microscope. The machine vision algorithm first located the centerline of the micropipette and each yolk. Then the micropipette was moved by $0.43\ mm$, $1.69\ mm$ and $2.96\ mm$ to align with the centerline of each yolk respectively. For each embryo, penetration was started upon each alignment. Figure 3 shows the trajectory of the penetration force of the first embryo. In the experiment, the average penetration time for each embryo was around 15 seconds.

IV. Summary

We have described the design and construction of a prototype micromanipulation system for automatic batch microinjection of the zebrafish embryos. The effectiveness of this prototype micromanipulation system has been demonstrated

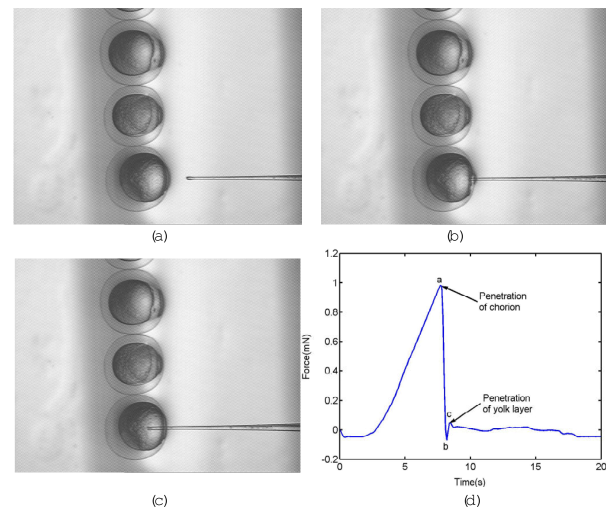

Fig. 3. Penetration of zebrafish embryo (a) before contact (b) contact (c) penetration (d) force trajectories of the penetration process.

in an experiment involving automatic identification and penetration of a group of zebrafish embryos. The experimental results demonstrate that the technique of position control with dynamic penetration-force feedback is practicable for automatic batch microinjection applications. This system will also improve the quality of existent microinjection process. For example, in the application of Intracytoplasmic Injection (ICSI), where a sperm is injected directly into an egg to achieve fertilization, the automatic microinjection system will ensures a high success rate by optimally controlling the penetration process. This reduces the risk of wasting eggs, especially when the egg is precious (such as in human ICSI).

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