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High-Throughput Fully Automated Microrobotic Zebrafish Embryo Injection

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***Abstract*— Injection of foreign materials (e.g., DNA, RNAi, sperm, protein, and drug compounds) into individual cells has significant implications in genetics, transgenics, assisted reproduction, and drug discovery. This video presents a microrobotic system for fully automated zebrafish embryo injection, which overcomes the problems inherent in manual operation, such as human fatigue and large variations in success rates due to poor reproducibility. Based on computer vision and motion control, the automated microrobotic system is capable of immobilizing a large number of zebrafish embryos into a regular pattern within seconds and injecting 15 embryos (chorion unremoved) per minute with a success rate, survival rate, and phenotypic rate all close to 100%.**

***Index Terms*— Microrobotic control, computer vision, cell injection, zebrafish embryos, high throughput, no-tail phenotype.**

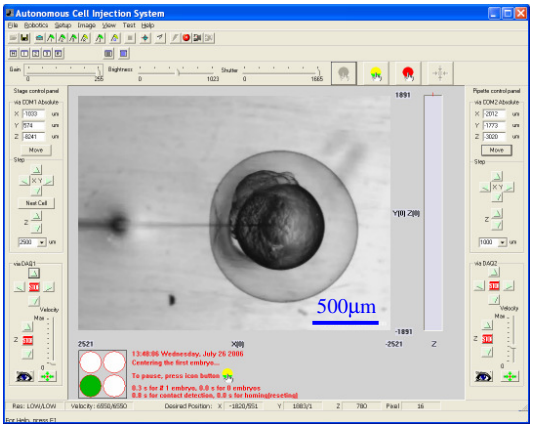

Fig. 1. Control program interface for zebrafish embryo injection.

EXTENDED ABSTRACT

Since the initial demonstration of semi-autonomous, visually servoed microrobotic cell injection [1], efforts from many research groups for automating cell injection have been continuous. The manipulated cell lines include mouse embryos, *Drosophila* embryos, epithelial cells, and zebrafish embryos. However, no fully automated systems exist that are capable of autonomously injecting a large number of cells without human intervention (e.g., locating features and destinations, switching from one cell to another, and injector alignment).

The zebrafish has become an important model organism for development and genetic studies as well as drug discovery due to the advantages of similarities in the major organs to humans, external fertilization and development, and short development period, to name just a few. Molecular and genetic analyses of zebrafish embryogenesis depend on the injection of foreign materials into early zebrafish embryos. For example, DNA injection for generating transgenic zebrafish lines, mRNA injection for overexpressing gene-products in zebrafish embryos, and the injection of antisense morpholino-modified oligonucleotides (morpholinos or MOs) for specifically inhibiting RNA splicing and/or translation *in vivo*.

For testing cellular responses to molecular targets and to obtain statistically significant data, the injection of thousands of cells needs to be conducted within a short time window (e.g., within 1.5hr after fertilization, before the 16-cell stage for zebrafish embryos). Manual injection is not only slow; the laborious task easily causes fatigue in injection technicians and hinders performance consistency and success rates.

Despite their relatively large size ($\sim$600μm without chorion), zebrafish embryos have a delicate structure and can be easily damaged. They are also highly deformable, making the automatic manipulation task difficult. Specific challenges in achieving automated, high-throughput zebrafish embryo injection include: (i) the ability to quickly (i.e., seconds) immobilize a large number of zebrafish embryos into a regular pattern; (ii) the ability to automatically and robustly identify cell structures for vision-based position control and account for size differences across embryos; and (iii) the ability to coordinately control two microrobots to achieve robust, high-speed zebrafish embryo injection.

In this video, a recently developed system [2] for zebrafish embryo injection (Fig. 1) is presented, featuring full automation, a high speed, fast sample immobilization, high survival rates, success rates, and phenotypic rates. The system employs two 3-DOF microrobots. An in-house developed embryo holding device is attached to the left microrobot. A vacuum pump provides negative pressure to immobilize embryos into regular patterns. A sharp-pulled injection micropipette is connected to the right microrobot via a micropipette holder. Two motion control cards are mounted on a host computer where control algorithms and image processing algorithms operate. Visual feedback is obtained through a camera mounted on an optical microscope. A computer-controlled pico-injector with a volume control resolution of sub-picoliter provides positive pressure for material deposition.

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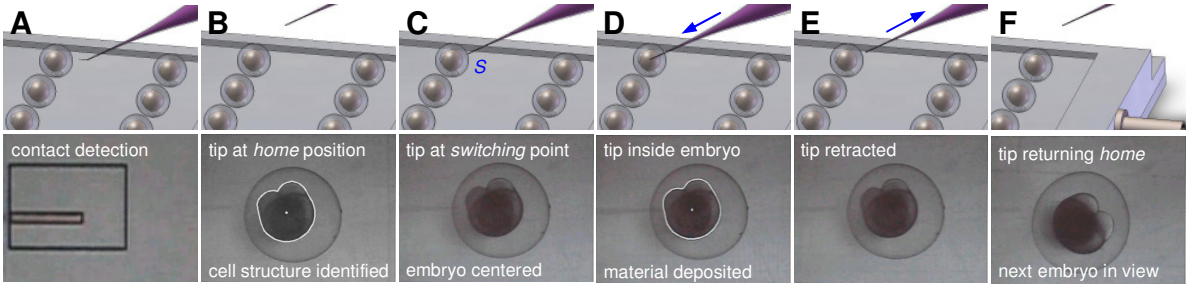

Fig. 2. Illustration of the automated injection flow. Left microrobot positions embryos, and right microrobot controls injection micropipette.

A vacuum-based embryo holding device was constructed for immobilizing individual zebrafish embryos (up to 100). Evenly spaced through-holes (diameter $\sim$400μm) are connected to a vacuum source via a backside channel. Upon dispersing a batch of embryos onto the device, a sucking pressure of 2-7InHg (fish strain dependent) enables each through-hole to trap a single embryo. The extra non-trapped embryos are flushed away from the device. Polycarbonate was chosen for fabricating the devices as it is optically transparent, biocompatible, inexpensive, and easy to machine.

A batch of zebrafish embryos, immobilized into a regular pattern on the embryo holding device, are placed on the left microrobot under the microscope. Fully automated injection starts with vision-based contact detection [3] to determine the vertical positions of the micropipette tip and the top surface of the embryo holding device and further, the relative vertical positions between the micropipette tip and the center of an embryo. The algorithm provides better robustness and detection accuracy (0.2μm) compared to focusing-based algorithms. Fundamentally, after the establishment of contact in the world frame, further vertical motion of the injection micropipette induces horizontal motion in the image plane. Before and after contact, the x-coordinates of the micropipette tip in the image plane result in a V-shaped curve against its downward displacement. The peak of the V-shaped curve represents the contact position along the vertical direction between the micropipette tip and the reference surface.

After contact detection, an embryo brought to the center of the field of view, and the embryo structures are recognized through real-time image processing. Simultaneously, the micropipette tip is moved by the right microrobot to a switching point, *S* that serves as an indicator of the boundary between inside and outside of an embryo and is determined through the recognition of embryo structures (Fig. 2). The micropipette tip penetrates the chorion and deposits materials at the desired location within the embryo. In this video, the deposition destination was chosen to be the cytoplasm center, where cytoplasm is defined as the combination of the yolk and the cell portion of a zebrafish embryo. Upon retreating out of the embryo, the micropipette tip is moved to a *home* position that is 1.4mm above the contact point, to prevent it from crashing into the next embryo. In the meanwhile, the next embryo is brought into the field of view, the structures are recognized, and the injection process is repeated until all embryos in the batch are injected.

The system permits on-line calibration of pixel sizes when positioning the first embryo within a batch. As pixel sizes vary with different microscope objectives, couplers, and cameras used for imaging, it is desired to eliminate the imaging-hardware dependence by conducting on-line calibration. The left microrobot brings the first embryo to the image center according to image-based visual servoing while the other embryos within the batch are still brought to the image center by position control.

Throughout the process, the left microrobot does not produce vertical motion while the right microrobot is servoed along three axes. For positioning each embryo and controlling the motion of the injection micropipette, PID control is employed for controlling both microrobots that are operated in parallel whenever possible. Parallel operation of the two microrobots is maximized to increase injection throughput. Transformations among the multiple coordinate frames are achieved during the operation of the system without requiring an off-line process.

For the ease of visually inspecting the injection effectiveness, fluorescent dyes (Rhodamine B, 100μM) were injected into 350 embryos. To quantify the efficacy of the system for re-capitulating mutant embryonic phenotypes, fluorescein-tagged morpholinos that target the gene no tail (ntl-MO, 5'-GACTTGAGGCAGGCATATTTCCGAT-3', 300nM, Gene Tools) were injected into additional 210 embryos. The cell injection system experimentally demonstrated the capability of injecting 15 zebrafish embryos per minute with a 98% survival rate, a 99% success rate, and a 98.5% phenotypic rate.

The video presents the complete experimental process of automated microrobotic zebrafish embryo injection including embryo immobilization, operation of the two microrobots, and experimental results to demonstrate that the microrobotic system is a reliable tool for determining gene functions and more generally, for facilitating large-scale molecule screening.

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