2014 IEEE International Conference on Robotics & Automation (ICRA)
Hong Kong Convention and Exhibition Center
May 31 - June 7, 2014. Hong Kong, China

# Development of a High Throughput Robot-aided Cell Injection System for Human Cells*

Yu Ting Chow[1], Shuxun Chen[1], Chong Liu[1], Shuk Han Cheng[2], Ronald A. Li[3], and Dong Sun[1]

***Abstract*—**Few of the current injection technologies can be applied to those human cells whose diameters are ranged about 10 − 25 μm only. This paper reports our most recent effort in developing a robot-aided microinjection system to solve the challenging problem of automated injection on human cells. A unique microfluidic cell holding chip is designed and fabricated to trap the single cells in the predefined docking area. Imaging processing technique is used to recognize automatically the target cells to be injected. A microrobot system equipped with a micropipette is used to perform the injection tasks on these target cells. Injection experiments on human embryonic stem cells (hESCs) (ranged about 17 − 25μm) are performed to demonstrate the effectiveness of the proposed microinjection system.

## I. Introduction

Study of specific cellular responses by transferring foreign intracellular factors into cells is a common biological practice. Delivery of intracellular factors such as DNA or RNA can be achieved biologically [1] (viral vector), chemically [2 − 3] (liposome), physically [4 − 6] (electric or laser pulse) and mechanically [7] (gene gun or microinjection). Among these methods, the biological and chemical method is notably performed based on mass production, with the efficiency that highly depends on different cell types. This method has difficulty to be used in some biological studies that require single cell analysis. The physical method utilizes electric or laser pulses to open a hole on cell membrane, allowing foreign facts to pass through the cell membrane. This method can be applied to single cell study but subject to passive diffusion of foreign factors, which may affect the delivery efficiency. The mechanical method, e.g., microinjection, employs a glass micropipette to penetrate through the cell membrane using a mechanical force and deliver liquid substance into cell. This method can guarantee direct transfer of foreign intracellular factors into a specific region inside the cell.

Microinjection has exhibited many applications in various areas such as in vitro fertilization, sperm injection and drug development. Currently, the majority of microinjection tasks are manually performed by skilled operators, supported by microscope. However, manual injections have much difficulty to succeed the injection tasks for those cells whose sizes are less than 25μm (e.g., human somatic cells). As the rapid development of biomedical research in recent years, there is a great demand to develop a new robot-aided technology that not only can eliminate human involvement to large extent for improving the microinjection efficiency but also can achieve microinjection on human cells whose diameters are usually ranged from 10μm to 25μm.

A typical microinjection system consists of three main parts, namely microscope, microinjector and micropipette, all working on a cell holder. With the advance of computer vision and automation technology, autonomous solution has been proposed to perform automated microinjection on large-scale biological samples (50μm-500μm). Sun *et. al.* developed an autonomous 3-DOF microrobot system to accomplish a cell injection task on mouse embryo (~55μm) [8]. Wang *et. al.* presented a vacuum-based embryo holding device in a fully automated robotic system for microinjection of Zebrafish Embryos (~500μm) [9]. Huang *et. al.* developed a robotic system with a combined position and force control to achieve automated batch injection of suspended embryo cells [10,11]. Liu *et. al.* developed an automated robotic microinjection system for delivering cell impermeable compounds into mouse zygotes (~100μm) [12]. Although significant efforts have been made to automate microinjection using robotic device in the past [13 − 17], an automated system for batch injection of human cells with much smaller size (<25 μm) has not been demonstrated yet.

To achieve microinjection on small single cells, it is necessary to integrate the microfluidic chip technology into the microinjection system [18]. Development of microfluidic chip technology has attracted considerable attentions in cell engineering study [19 − 22]. Microfluidic chip technology has provided a new way for single cell handing and analysis [24], such as cell-to-cell interaction [24], cell sorting [25] and cell fusion [26]. Micro and nano-scale engineering approaches have been developed to enhance our understanding of cell behavior in a fast and precise manner [27]. These approaches lay a solid base for the development of a new microfluidic cell holding chip for the microinjection system.

This paper reports our recent development of a vision-based automated cell injection system for single human cells whose diameters are smaller than 25 μm. A uniquely designed microfluidic chip was fabricated and used as a vacuum-based cell holder to locate single cells in predefined locations. Imaging processing was applied to recognize the target cells precisely. A microinjection system with a micropipette, controlled by a closed-loop motion controller, was employed to perform injection process. This cell injection system exhibits the following advantages. First, it is able to accomplish high volume microinjection with high accuracy, as supported by a uniquely designed cell holder structure that

* This work was supported by a grant from the Theme-based Research Scheme (T13-706), and a grant from Research Grants Council of the Hong Kong Special Administrative Region, China (Reference No. CityU 121513).

[1]Department of Mechanical and Biomedical Engineering, City University of Hong Kong, Hong Kong, China (e-mail: yutchow2@gapps.cityu.edu.hk, shuxuchen2-c@my.cityu.edu.hk and medsun@cityu.edu.hk).

[2]Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, China (e-mail: bhcheng@cityu.edu.hk).

[3]Stem Cell & Regenerative Medicine Consortium, and Departments of Medicine and Physiology, LKS Faculty of Medicine, University of Hong Kong, Hong Kong. (email: lironald@me.com)

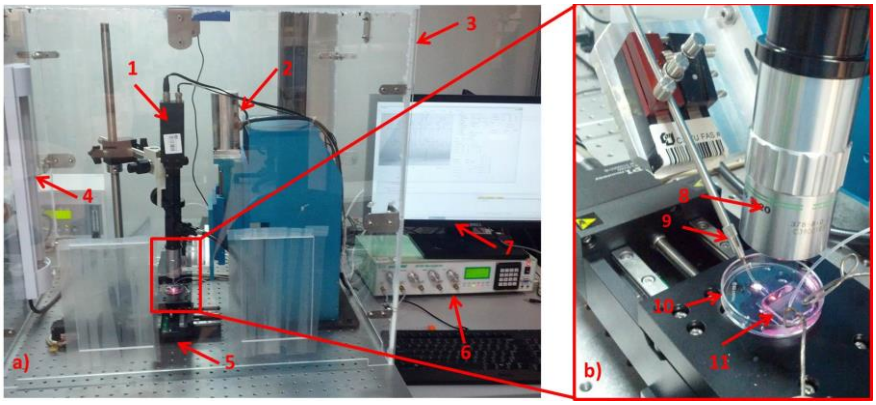


Figure 1. a) Cell injection system setup. 1) CCD camera, 2)Z-Linear table, 3) Injection chamber, 4) UV Lamp, 5) XY-micropositioning stage, 6) microinjector, 7) Computer and 3-axis controller. b) Close-up of the setup. 8) microscope, 9) Micropipette, 10) Petri dish and 11) Cell holder.

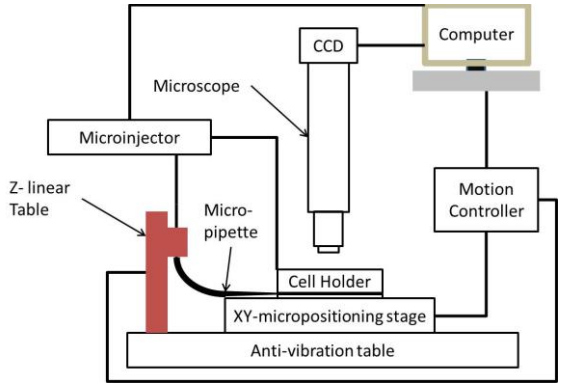


Figure 2. The schematic diagram of cell injection system setup.

can dock the target cells in a traceable manner. Second, the cell to be injected can be as small as 10 μm, hence making the injection system applicable to human cells. Third, the cell holder chip can be integrated with other microfluidic chips, which allows further operations without removing the injected cells from the microfluidic platform.

## II. System Design

In view of the tiny size of human cells, the automated microinjection system to be developed must be able to perform high accuracy and high speed batch injection with submicron resolution. In this section, we will introduce our developed new system setup, which included a cell injection system with a cell holding chip for trapping cells in the desired location, a cell recognition system using edge-based template matching algorithm, and a precise motion control system.

### *A. Injection System Setup*

Fig. 1 illustrates the developed setup of the automated robot-aided cell injection system, and Fig. 2 illustrates a diagram of the setup. The whole system consists of three modules: a computer vision module, a motorized stage control module and an executive module.

The computer vision module contains a vision system that includes an optical microscope, a lighting system, a CCD camera and an image grabber. The microscope is composed by a 20X objective (Mitutoyo, Japan) and an observation tube (Infinity Tube, USA). The lighting source is provided by an

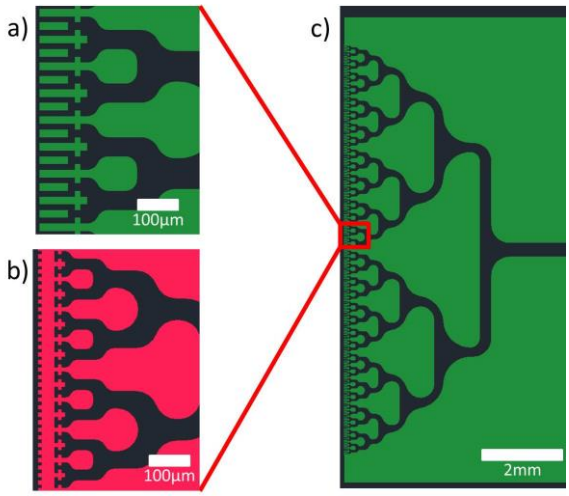


Figure 3. Flow direction when negative pressure is applied. Cell docking zone located at the edges of the chip.

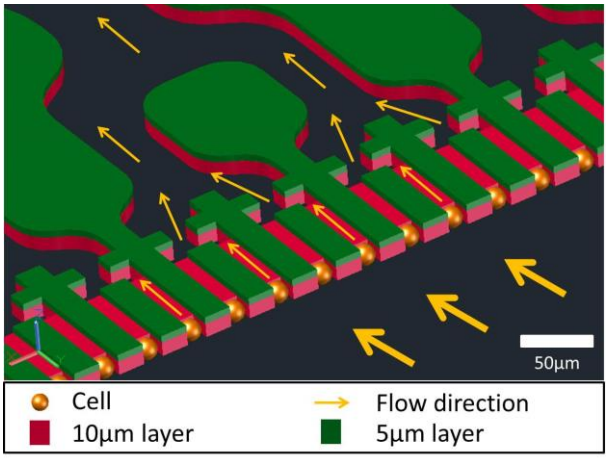


Figure 4. The schematic of the cell holder design. a) 5μm layer. b) 10μm layer. c) The overall view of the design.

illuminator (PL-800, Fiber-Lite). A CCD camera (STC-700, SENTECH) is mounted on the other end of the observation tube instead of eyepiece. The image is captured through a PC2-Vision frame grabber (OC-PC2M-VUM00, DASAL

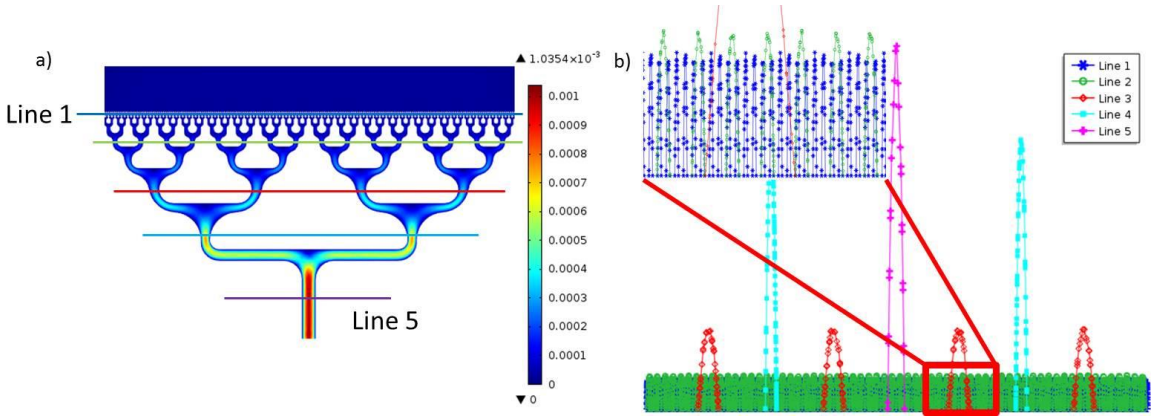


Figure 5. Simulation results. a) Velocity distribution surface. b) Velocity distribution curve along line 1 to 5. Enlarged view for observing line 4 and 5 (Top left corner).

Corp.) and processed by a windows based image processing library named Saparal™ Essential.

The motorized stage control module contains a X-Y micro position stage (PI M-L01, Physik Instrumente Co., Ltd) and a Z-axis linear table (KR30H06A, THK CO., LTD), driven by a 3-axis motion controller (DCT0040, Dynacity Tech. Ltd). All the X, Y, Z axes have resolutions of 1μm.

The executive module is composed of a micropipette, a microfluidic cell holder, and a pressure based microinjector (IM-300, NARISHIGE). The micropipette is mounted on the Z-axis linear table, and the microfluidic cell holder is placed on the X-Y stage. The microinjector is connected to the micropipette through a tube, providing a positive pressure to deliver liquid substance. The microinjector is also connected to the microfluidic cell holder, providing a negative pressure to immobilize suspending cells.

*B. Microfluidic Device*

To achieve high throughput cell injection, a microfluidic cell holding chip is designed to trap cells at the predefined positions. The cell holding chip comprises of 256 polydimethylsiloxane (PDMS) cell arrays at the end of bisection-tree-like channel. The chip is composed of two layers of structure, 5μm thick channel supporting layer (Fig. 3a), and 10μm thick cell docking layer (Fig. 3b). The width of the trapping channel is 15μm. A schematic showing how cell is trapped is given in Fig. 4. The trapping force is generated by driving fluid flow from the cell docking zone to the outlet. In order to generate the suction force, a negative pressure was applied to the outlet, so as to create current flow towards the cell docking zone. Then, cells will experience a fluid drag force by the flowing culture medium. Eventually, the cell will be located regularly in the docking area. The channel-supporting layer only allows fluid to flow thorough, but the cells with a diameter larger than 5μm cannot pass. By using this holding chip, cells can be trapped and located easily in the cell holder.

Fabrication of the microfluidic holding chip utilizes the soft lithography technology. The PDMS channel can be obtained through the molding of reverse structure of the master. To fabricate the master, a 3-inch silicon wafer was spin-coated with 5μm thick SU-8 photoresist (GM1050, Gersteltec Sarl). Then the coated wafer was prebake on a hotplate (AccuPlate, Labnet) at 95°C rising from room temperature for 3 minutes. After heating, the wafer was exposed to UV light with the mask of the channel-supporting layer on surface. The exposed wafer was baked at 80°C for 2 minutes for crosslinking the exposed SU-8. The exposed area was developed by immersing in SU8 developer (DRGM, Gersteltec Sarl). The fabrication of the 10μm thick cell-docking layer was the same as that for the first layer. The mask of cell docking layer was precisely aligned on the channel-supporting layer using an aligner.

To fabricate the microfluidic chip, PDMS, mixing with curing agent with 1:10 ratio, was poured on the master mold and degassed in a vacuum desiccator. Then, it was heated in an oven with $80^0$C for 2 hours. The cured PDMS was peeled off from the mold and was cut on a micromanipulation table.

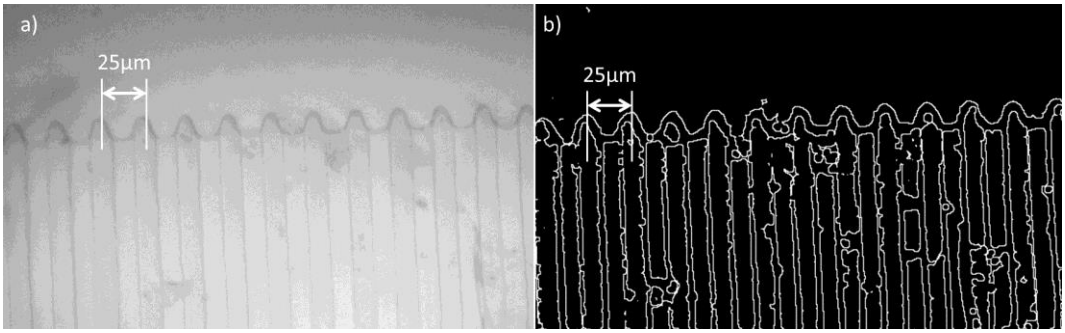


Figure 6. The cell holder images before and after filtering. a) Raw image. b) Processed image.

TABLE I. IMAGE PROCESS FLOW FOR RECOGNITIZING CELL

| Process step | Image Process Techniques Used |
|---|---|
| 1 | Gaussian Filter |
| 2 | Median Filter |
| 3 | Sobel Edge detector |
| 4 | Closing |
| 5 | Thining |
| 6 | Edge Template Matching |

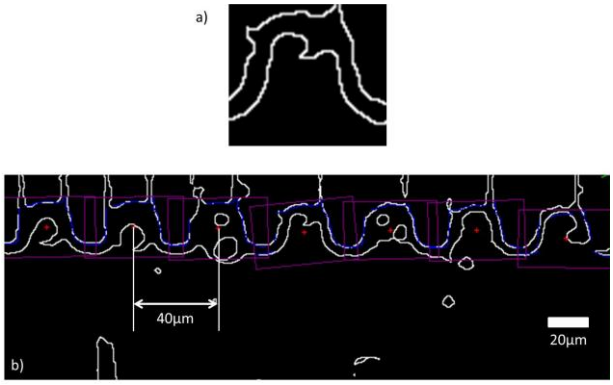


Figure 7. Cell traps recognition using the edge template matching algorithm. a) The template image. b) Cell trap position after recognition.

The cutting process was conducted under a microscope with 5X objective. The surface of the processed PDMS and a clean cover slide were then plasma activated and bonded together to form irreversible seal.

Simulation was conducted using Comsol Multiphysics finite element software to determine whether the suction force on each trap was uniform. MEMS module with incompressible Navier-Stoke was used to simulate the flow behavior of the designed cell holder. The simulation results are shown in Fig. 5. As shown in Fig. 5(a), the velocity distribution on the whole cell holder was uniform. The velocity distribution along 5 lines in Fig. 5(a) is plotted in Fig. 5b. It is seen that the flow velocity for each channel was the same. This confirmed that the bisection-tree-like channel network was able to achieve uniform suction force, when negative pressure was applied to outlet.

## III. AUTOMATED CELL INJECTION

Automated cell injection can be divided into two main processes: image processing technique used for target cell

### *A. Image Processing*

After cells were trapped in cell docking area of microfluidic chip, the images were captured by scanning across the docking area with the programmed X-Y micropositioning stage. A sequence of spatial filter was used to eliminate the noise. A low-pass Gaussian filter and median were applied to the captured image to remove the high frequency noise and pepper noise respectively. The edge information of each channel was extracted by Sobel operation and a binary image was obtained. Small discontinuities were connected on the binary image and the boundary of the image was acquired by applying thinning

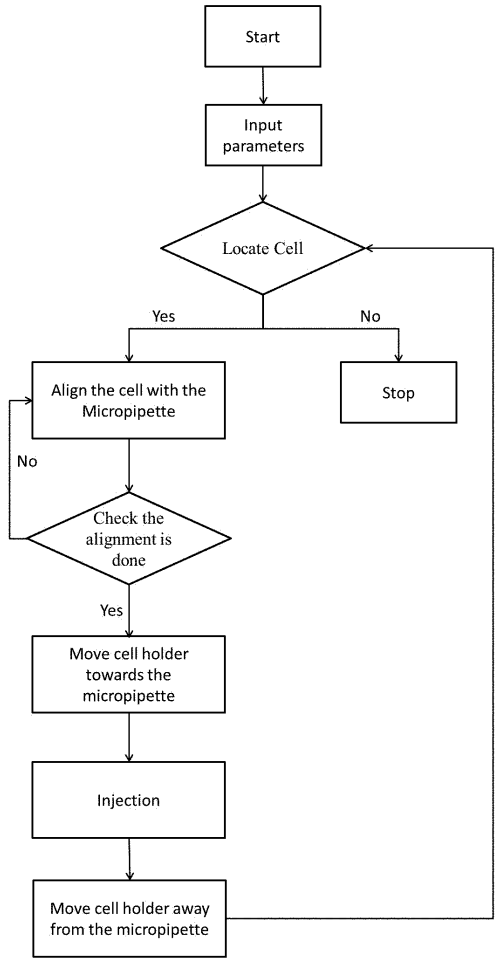


Figure 8. Flow diagram of automated cell injection.

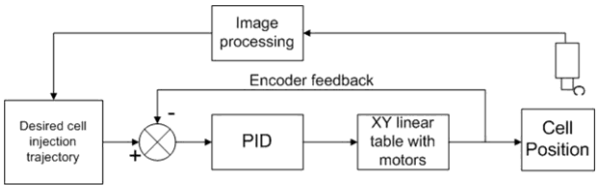


Figure 9. The visual base position control architecture of the microinjection system.

operation. Fig. 6 illustrates images before and after processing. The procedures used for image processing operation are given in Table 1.

The edge template matching algorithm was used to locate the cell docking position. First, a typical single cell trap element inside the cell holder was manually selected. The template image was then processed by following the procedures as listed in Table 1. When the correlation between the input image and the template was larger than 70%, the center of the matched pattern was recognized as the cell docking position. Fig. 7 (a) illustrates a template image, and Fig. 7(b) illustrates the result of the cell docking position recognized by using the template image. Fig. 7(b) illustrates the result of the cell docking position recognized by using the template image. All the cell traps are located in Fig. 7(b). This shows detection can be stable even with high background noise.

### B. *Automatic Cell Injection*

Before injection, the tip position of the micropipette and the image of the edge template were input to the system first. The origin of the image plane was set at the tip position of the micropipette. After the cell docking position was recognized, the cell trajectories in X and Y axes were generated, based on the relative position between the micropipette tip position and the cell docking position. Then, the following steps were implemented. First, the position of the cells is determined by template matching algorithm. Then, the micropipette was aligned with the first cell on the left hand side in X-axis. The cell was moved in Y-axis according to the calculated trajectory. The microinjector injected the material into the micropipette, with preset injection pressure and time. After the injection was completed, the cell holder moved away from the tip of the micropipette and back to the previous position. Template matching is executed again to determine the next target cell location. The flow diagram of such an automated cell injection process is shown in Fig. 8. All motions of X-Y positioning stage were controlled by a motion controller, utilizing the feedback from the motor encoders and the CCD camera captured image. Fig. 9 shows the visual based position control architecture.

## IV. Experiments and Results

Experiments of microinjection on hESCs were performed to evaluate the performance of the proposed robot-aided microinjection system. Microinjection of intracellular factors into hESCs can be used for facilitating the investigation on cell differentiation.

The hESC culture method was previously described in detail [25]. Prior to the experiments, the microfluidic cell holding chip was filled with 70% ethanol and exposed under UV for 30 min for sterilization. Then the chip was washed twice with the cell culture medium. Air bubbles were removed from bisection-tree-like channel by placing the chip in a vacuum desiccator. The chip was finally fixed in a 35 mm petri dish, which was placed on the X-Y micropositioning stage. The microinjector was connected to the outlet of the chip through polyethylene tubing. The human cells were obtained with 0.05% trypsin–EDTA treatment and suspended in culture medium.

At the beginning of experiment, the micropipette which loaded with liquid substance was first inserted into the 15μm gap between PDMS and bonded cover glass slip by controlling the Z-aixs linear table and X-Y stage manually. The petri dish was filled with cell culture medium until the cell holding chip was fully immersed. Then 20μl hESCs (~1000 cells/μl) were placed into petri dish and trapped at cell docking area of the cell holding chip, under the control of negative pressure applied by microinjector. This process takes about a few minutes. The residue cells were removed by pipette to carefully suck away medium. Figure 10 shows the result of cell trapping by the microfluidic chip.

Figure 11 illustrates the automated microinjection process. After imaging processing, the target cell was aligned with the micropipette in X-axis as shown in Fig. 11a. Then the target cell was driven towards micropipette tip in Y-axis. The microinjector applied a positive pressure to the micropipette

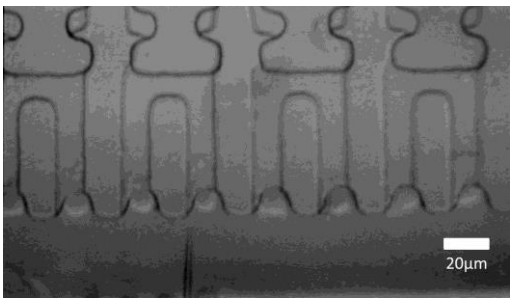


Figure 10. Experimental results. The cells are able to be trapped in the cell holder.

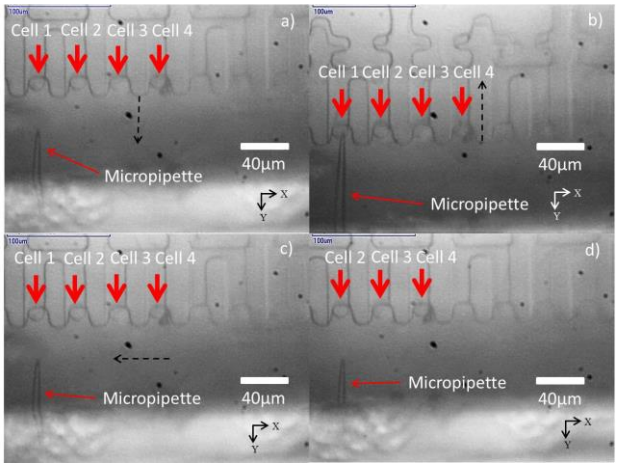


Figure 11. Automated cell injection procedures. a) Target cell was aligned with micropipette tip in X-axis. b) Cell was injected. c) Injected cell was pulled back. d) New cell was aligned with micropipette tip.

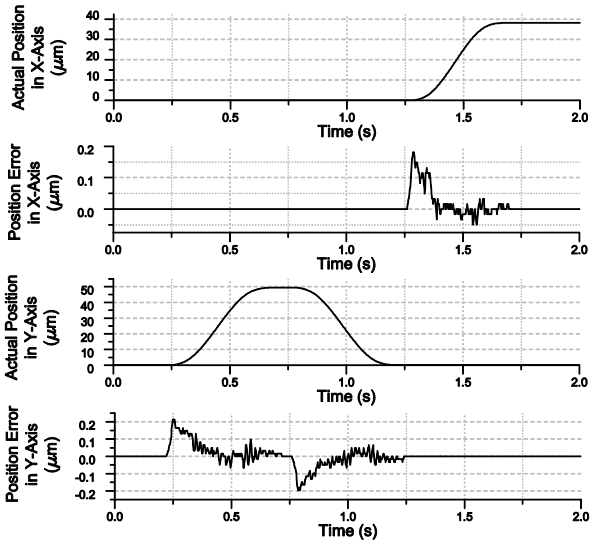


Figure 12. Motion performance of the X-Y stage during cell injection. a) Actual position in X-axis. b) Position error in X-axis. c) Actual Position in Y-axis. d) Position error in Y-axis.

and injected liquid substance into cells, as shown in Fig. 11b. After injection, the cell moved back with the X-Y position stage and the next target cell was aligned with the micropipette tip in X-axis, as shown in Fig 11c and Fig. 11d. The above procedures were repeated until all the cells were injected. Figure 12 illustrates the motion trajectories in X-axis

and Y-axis, demonstrating that the position control algorithm exhibited satisfactory tracking performance for such micromanipulation.

In the experiment, the average time for the automated cell injection process, including moving the cell holder toward the micropipette, performing injection, moving the cell holder away from the micropipette and switching to the next target cell (one cycle), was about 2 seconds in total. Thus, the average throughput was 30 cells per minute.

## V. Conclusion

In this paper, a robot-aided automated microinjection system for human cells with much smaller size (10 – 25μm) compared to embryos (>50μm) was developed. The developed cell injection system was able to perform automated batch cell injection with the help of microfluidic chip and image processing techniques. A microfluidic cell holding chip was particularly designed and fabricated to hold the target cells at the predefined cell docking positions, which were then located by image processing technique. The target cells were injected under manipulation of a microrobotic injector, which adopted a visual based position control algorithm. Microinjection experiments on hESCs were performed to demonstrate the effectiveness of the proposed approach. The proposed technology can largely improve both efficiency and capability of the current cell injection industry.

## Acknowledgment

The authors wish to thank Dr. Chi-wing Kong from the Stem Cell and Regenerative Medicine Consortium at the University of Hong Kong for providing the hESCs, as well as for his invaluable assistance with cell culture.

## References

[1] P. D. Robbins and S. C. Ghivizzani, "Viral Vectors for Gene Therapy," *Pharmacology & Therapeutics*, vol. 80, no. 1, pp. 35 – 47, 1998.

[2] F. Graham and A. Van der Eb, "Transformation of rat cells by DNA of human adenovirus 5," *Virology*, vol. 54, no. 2, pp. 536–539, 1973.

[3] T. Alexakis, D. Boadi, D. Quong, A. Groboillot, I. O'neill, D. Poncelet, and R. Neufeld, "Microencapsulation of DNA within alginate microspheres and crosslinked chitosan membranes for in vivo application," *Applied biochemistry and biotechnology*, vol. 50, no. 1, pp. 93–106, 1995.

[4] M. Costa, M. Dottori, K. Sourris, P. Jamshidi, T. Hatzistavrou, R. Davis, L. Azzola, S. Jackson, S. M. Lim, M. Pera *et al.*, "A method for genetic modification of human embryonic stem cells using electroporation," *Nature protocols*, vol. 2, no. 4, pp. 792–796, 2007.

[5] I. B. Clark, E. G. Hanania, J. Stevens, M. Gallina, A. Fieck, R. Brandes, B. O. Palsson, and M. R. Koller, "Optoinjection for efficient targeted delivery of a broad range of compounds and macromolecules into diverse cell types," *Journal of biomedical optics*, vol. 11, no. 1, pp. 014034–014034, 2006.

[6] D. J. Stevenson, F. J. Gunn-Moore, P. Campbell, and K. Dholakia, "Single cell optical transfection," *Journal of the Royal Society Interface*, vol. 7, no. 47, pp. 863–871, 2010.

[7] Y. Zhang, "Microinjection technique and protocol to single cells," *Protocol Exchange*, Nov 2007.

[8] Y. Sun and B. J. Nelson, "Biological cell injection using an autonomous microrobotic system," *The International Journal of Robotics Research*, vol. 21, no. 10-11, pp. 861–868, 2002.

[9] W. Wang, X. Liu, D. Gelinas, B. Ciruna, and Y. Sun, "A fully automated robotic system for microinjection of zebrafish embryos," *PLoS One,* vol. 2, 2007.

[10] H. B. Huang, D. Sun, J. K. Mills, and S. H. Cheng, "Robotic cell injection system with position and force control: toward automatic batch biomanipulation," *Robotics, IEEE Transactions on*, vol. 25, no. 3, pp. 727–737, 2009.

[11] H. B. Huang, D. Sun, J. K. Mills, W. J. Li, and S.H. Cheng, "Visual-Based Impedance Control of Out-of-Plane Cell Injection Systems," *Automation Science and Engineering, IEEE Transactions on,* vol. 6, pp. 565-571, 2009.

[12] X. Liu and Y. Sun, "Microfabricated glass devices for rapid single cell immobilization in mouse zygote microinjection," *Biomedical Microdevices,* vol. 11, pp. 1169-1174, 2009.

[13] Y. Xie, D. Sun, C. Liu, H. Y. Tse, and S. H. Cheng, "A Force Control Approach to a Robot-assisted Cell Microinjection System," *The International Journal of Robotics Research,* November 13, 2009 2009.

[14] Y. Xie, D. Sun, C. Liu, H. Y. Tse, and S. H. Cheng, "Force sensing and manipulation strategy in robot-assisted microinjection on zebrafish embryos," *IEEE/ASME Trans. on Mechatronics*, vol. 16, no. 6, pp. 1002-1010, December 2011

[15] K. Sakaki, N. Dechev, R. D. Burke, E. J. Park, "Development of an autonomous biological cell manipulator with single-cell electroporation and visual servoing capabilities," *IEEE Trans. Biomed. Engineering,* 56(8): 2064-2074 (2009)

[16] Z. Lu, C. Y. Chen, J. Nam, R. Ge, and W. Lin, "A micromanipulation system with dynamic force-feedback for automatic batch microinjection," *J. of Micromechanics and Microengineering*, vol 17, no. 2, pp. 314, 2007.

[17] U-Xuan Tan, Win Tun Latt, Cheng Yap Shee, and Wei Tech Ang. A low-cost flexure-based handheld mechanism for micromanipulation. *Mechatronics, IEEE/ASME Transactions on*, 16(4):773–778, 2011.

[18] A. Adamo and K. F. Jensen, "Microfluidic based single cell microinjection," *Lab on a Chip*, vol. 8, no. 8, pp. 1258–1261, 2008.

[19] H. Lee, Y. Liu, D. Ham, and R. M. Westervelt, "Integrated cell manipulation system-CMOS/microfluidic hybrid," *Lab on a Chip*, vol. 7, no. 3, pp. 331–337, 2007.

[20] S. Kobel, A. Valero, J. Latt, P. Renaud, and M. Lutolf, "Optimization of microfluidic single cell trapping for long-term on-chip culture," *Lab on a Chip*, vol. 10, no. 7, pp. 857–863, 2010.

[21] A. M. Skelley, O. Kirak, H. Suh, R. Jaenisch, and J. Voldman, "Microfluidic control of cell pairing and fusion," *Nature methods*, vol. 6, no. 2, pp. 147–152, 2009.

[22] D. Di Carlo, N. Aghdam, and L. P. Lee, "Single-cell enzyme concentrations, kinetics, and inhibition analysis using high-density hydrodynamic cell isolation arrays," *Analytical chemistry*, vol. 78, no. 14, pp. 4925–4930, 2006.

[23] M. Gel, S. Suzuki, Y. Kimura, O. Kurosawa, B. Techaumnat, H. Oana, and M. Washizu, "Microorifice-Based High-Yield Cell Fusion on Microfluidic Chip: Electrofusion of Selected Pairs and Fusant Viability," *NanoBioscience, IEEE Transactions on,* vol. 8, pp. 300-305, 2009.

[24] X. Gou, H. Han, S. Hu, A.Y.H. Leung, and D. Sun, "Applying combined optical tweezers and fluorescence microscopy technologies to manipulate cell adhesions for cell-to-cell interaction study," *IEEE Trans. on Biomedical Engineering*, vol. 60, no. 8, pp. 2308-2315, August 2013.

[25] X. Wang, S. Chen, M. Kong, Z. Wang, K. D. Costa, R. A. Li, and D. Sun, "Enhanced cell sorting and manipulation with combined optical tweezer and microfluidic chip technologies," *Lab on a Chip*, 2011,11(21): 3656-3662.

[26] X. Wang, X. Gou, S. Chen, X. Yan, and D. Sun, "Cell manipulation tool with combined microwell array and optical tweezers for cell isolation and deposition," *Journal of Micromechanics and Microengineering*, 2013, 23(7): 075006.

[27] E. Ghafar-Zadeh, J. R. Waldeisen, and L. P. Lee, "Engineered approaches to the stem cell microenvironment for cardiac tissue regeneration," *Lab on a Chip,* vol. 11, pp. 3031-3048, 2011.