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바이오 셀 조작용 지능 로봇 시스템

An Intelligent Robotic Biological Cell Injection System

심재홍* · 조영임** · 김종형***

Jae-Hong Shim, Young-Im Cho and Jong-Hyeong Kim

* 한국산업기술대학교 제어계측공학과

** 평택대학교 컴퓨터과학과

*** 서울산업대학교 기계설계자동화공학부

요 약

최근 바이오 관련산업의 발전과 함께 바이오 장비 및 장치들에 대한 연구 및 개발이 활발하게 진행되고 있다. 특히 바이오 세포 조작관련 연구들이 많이 진행되어 오고 있다. 일반적으로 바이오 세포들에 대해 기계적인 엔드 이펙터들이 조작을 위해 접촉될 때 과도한 힘이 발생될 경우가 발생하며 이런 힘들에 의해 세포막이나 조직들이 피해를 입을 수 있다. 본 논문에서는 상기 문제들을 극복하기 위해 바이오 세포 조작을 위한 새로운 시스템을 제안하였다. 제안된 시스템은 내장된 힘 센서를 이용하여 바이오 세포와 엔드 이펙터간의 발생 힘을 측정할 수 있다. 또한, 비전기술을 이용하여 엔드 이펙터의 피펫 팁을 바이오 세포막까지 정확하게 가이드 할 수 있다. 결과적으로 제안된 시스템은 바이오 세포에 피해를 주지 않고 안전하게 조작이 가능하다. 제안된 기술을 이용하여 실제 시작품을 제작하여 다양한 실험을 수행한 결과 향후 DNA 조작과 같은 바이오 세포 조작용 정밀 인젝션 시스템으로의 사용 가능성을 보여 주었다.

Abstract

Recently, instruments and systems related on biological technology have been enormously developed. Particularly, many researches for biological cell injection have been carried out. Usually, excessive contact force occurring when the end-effector and a biological cell contact might make a damage on the cell. Unfortunately, the excessive force could easily destroy the membrane and tissue of the cell. In order to overcome the problem, we proposed a new injection system for biological cell manipulation. The proposed injection system can measure the contact force between a pipette and a cell by using a force sensor. ·Also, we used vision technology to correctly guide the tip of the pipette to the cell. Consequently, the proposed injection system could safely manipulate the biological cells without any damage. This paper presents the introduction of our new injection system and design concepts of the new micro end-effector. Through a series of experiments the proposed injection system shows the possibility of application for precision biological cell manipulation such as DNA operation.

Key words : Micro end-effector, Pipette, Biological cell, Injection, Manipulation, Force sensor

1. Introduction

The bio cells' tissue or membrane is very fragile and slippery. Therefore, it needs skillful injection technique. Many researchers have been studied it for the past years[1-3].

Usually, injection tip system for biological cell manipulation has been reported to have so simple structures. And there is no force sensing mechanism. It can only control the positional motion of the micro end-effector or micro injection tip. When the micro end-effector contacts with a biological cell, it can hardly understand the physical characteristics of the cell, for example, the rigidity of the cell membrane. Due to these reasons, there exist lots of problems such as slippery and destruction of the cell membrane and damage of the pipette tip etc[4-5].

To overcome the problems, in this paper, we have developed a new biological cell injection system. It can measure the distance between the cell and injection tip by computer vision system. In addition, it can obtain the information of the contact force occurred when the micro end-effector contacts with the cell. These information can offer the physical characteristics in the micro world where the pipette tip contacts with the cell.

This paper is composed of six chapters. In chapter 2, we stated the problems of the existing micro end-effector. And we described the design concepts of a new micro end-effector for biological cell manipulation. In chapter 3, in order to obtain the best design data, we analyzed the structures of the micro end-effector using the finite element analysis software, 'ANSYS'. In chapter 4, we manufactured the biological cell manipulation system and performed the injection experiments. Through a series of experiments, we knew that the pro-

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posed micro end-effector can safely manipulate the fragile biological cells.

2. Design Concept of End-effector for Sensing Contact Force

2.1 Characteristics and Structures of Existing Micro End- effector for Biological Cell Manipulation

Existing micro end-effectors for biological cell manipulation used the simple structured pipette tip[6-7]. Most of them didn't equipped with a force sensor. Therefore, the existing micro end-effector could control only its position under the optical microscope. Usually, excessive contact force occurring when the end-effector and a biological cell contact might make a damage on the cell. Unfortunately, the excessive force could easily destroy the membrane and tissue of the cell. Also, some of the existing micro end-effectors carried out biological cell manipulation by using visual information[9]. But, they couldn't effectively manipulate the cells. Because of these conditions, it could little understand the physical property occurred when the micro end-effector contacted with the biological cell. We introduce a new micro injection end-effector that can solve these problems at next chapter 2.2.

2.2 Design of a New Micro End-effector

Fig. 1 shows the structure of the proposed micro injection end-effector capable of sensing the contact force. The micro injection end-effector is composed of pipette tip, tip holder, capillary holder and strain gauges as shown in Fig. 1 The diaphragm had strain gauges that measure the compressive force which is transmitted via the pipette tip. It is attached under the tip holder. The pipette tip is mounted at the center hole of the strain gauge of the diaphragm. The four strain gauges can be attached to the diaphragm as shown in Fig. 1. It can measure three kinds of forces such as Fz, Mx, My. Fz means two types of force information occurring when the pipette tip contact(contact force) and penetrate the cell membrane(penetration force).

As the cell membrane is so slippery, the pipette tip may easily slip on the surface of the cell membrane when it contact to the cell. Mx and My mean the moment information generated when it slipped. This moment information offer the wrong operation of the pipette tip. For example, when the pipette tip is injected into the cell correctly, the perpendicular repulsion force Fz is only measured. On the other hand, when the pipette tip is injected into the cell incorrectly, the cell membrane gets to be bent. The magnitude of Fz is much smaller than Mx and My. By using these information, we can improve the manipulability of the biological cell.

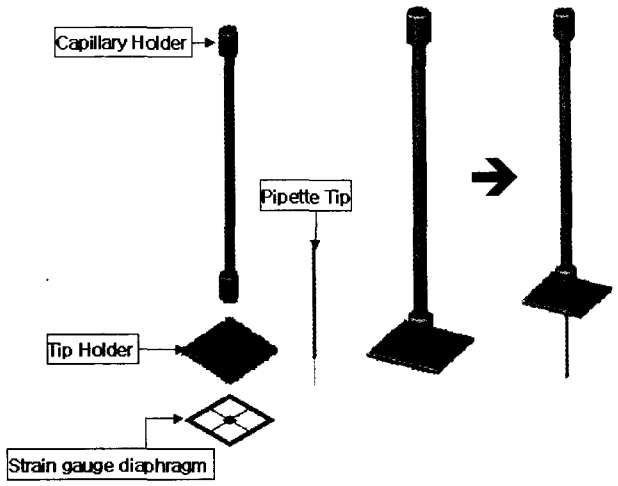


Fig. 1. Proposed micro end-effector for bio-cell injection.

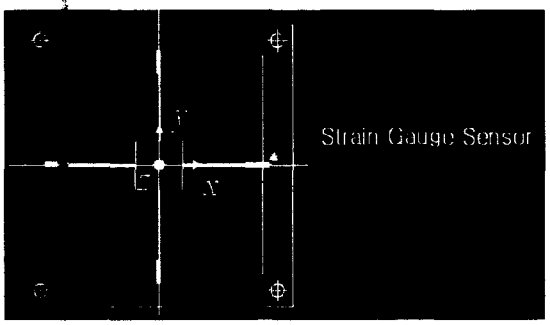


Fig. 2. Diaphragm attached strain gauge.

3. Design and Analysis of The Proposed Micro End-effector

We analyzed two dimensional displacement and stress of the diaphragm by using the finite element method. It generated stress concentration on the frame that encountered thin nodes. In this paper, we used the ANSYS that is a famous commercial finite element software.

The materials of diaphragm is stainless steel(SUS304). We set the Young's modulus: 198 GPa, Poisson's rate: 0.3. We analyzed the plane elasticity that changed thickness. When put the force at the perpendicular direction Z, displacement is generated at direction of X, Y. Using these data, the whole stresses of the diaphragm is computed.

We perform the analysis by the finite element method as follows;

1) We set the static force 0.1 mN in the middle of the diaphragm by changing the thickness of the node T; 1.5 mm, 2.5 mm, and 3.5 mm. Then, we observed principle stress.
2) We set the static force 0.1 mN in the middle of the diaphragm to change the thickness of the diaphragm; 0.1 mm and 0.12 mm. And, we observed principle stress.

Fig. 4 and Fig. 5 show the result of the stress

analysis. We know that the hinge is the part of stress concentration.

The maximum stress of Fig. 4(a) is 0.272 N/mm and that of Fig. 4(b) is 0.186 N/mm. The maximum stress of Fig. 5(a) is 0.394 N/mm and that of Fig. 5(b) is 0.271 N/mm. From the result of the analysis, we find out that the thinner thickness of the node and the diaphragm are, the bigger its stress is. And we know that the stress is concentrated at the hinge which thin rim and base meet. Therefore, to increase the sensitivity of the strain gauge sensor, the sensor must be attached to the hinge. And we decided the thickness of the node, T, as 0.3 mm, because the width of the semiconductor type strain gauge is 0.3 mm.

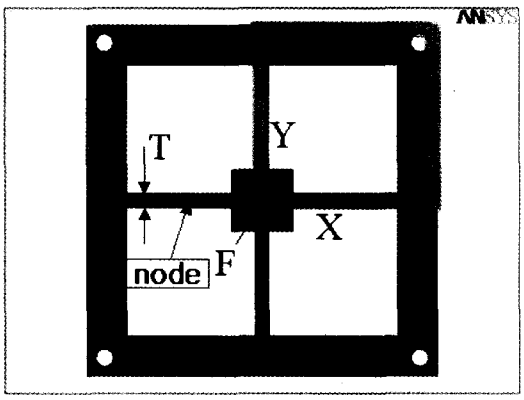
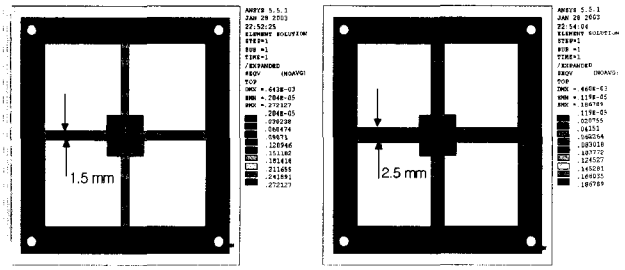
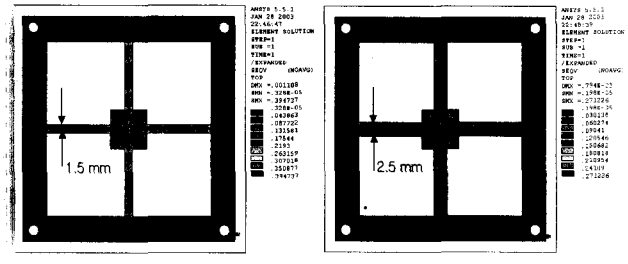


Fig. 3. Initial finite element mesh in analysis.



(a) (b)

Fig. 4. Stress in case of applying the 0.1mN force on 0.12t diaphragm normally.



(a) (b)

Fig. 5. Stress in case of applying the 0.1mN force on 0.1t diaphragm normally.

4. Experiments and Discussion for Biological Cell Manipulation

4.1 System Configuration

The system has two parts as shown in Fig. 6. There is vision processing part and motion control part with force sensing. These two parts are connected by RS232 serial communication each other. Vision processing part is composed of CCD camera, optical microscope, image capture board(frame grabber), and vision processing computer. Motion control part has force sensing mechanism and 2 D.O.F. manipulator.

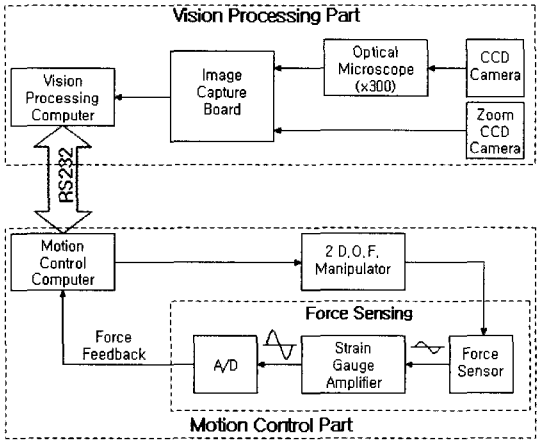


Fig. 6. System controller block-diagram

Two personal computers are used; the one is for motor control and force signal conditioning and the other is for vision processing.

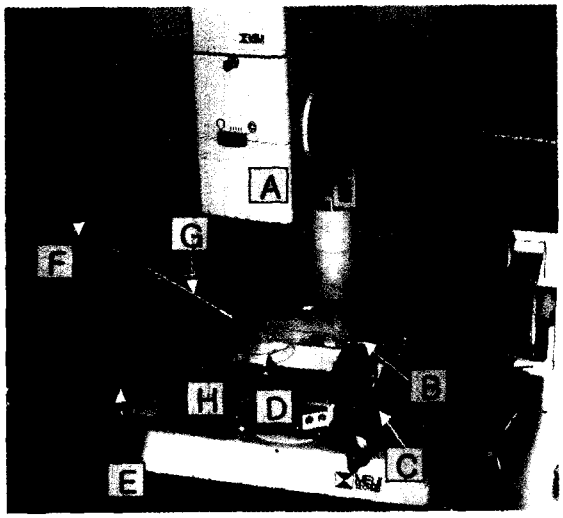
Motion control of the new micro end-effector is accomplished by 2 D.O.F. actuators. The one is rotational motion, and the other is linear. It composed of two stepping motors. Rotational actuator adjusts the angle of injection and the linear motion actuator is used to force the pipette penetrate biological cells. The 2 D.O.F. actuators are controlled by MEI motion control board in PC. The Strain gauge sensor is connected to the bridge-circuit box and the box is connected with the strain gauge amplifier. The analog output signal in the strain gauge amplifier is connected to the MEI board which performs A/D conversion. This converted A/D data is used to control the 2 D.O.F. actuators. In this way, the micro end-effector is controlled by using the information of the force sensor.

A holding tip is attached to the capillary holder's gripper. The tip is used to hold the biological cell by using the vacuum force. Capillary holder is connected to the holding device that is called CellTram Oil(Effendorf Co., Ltd.).

A CCD camera is mounted on the top of the optical microscope. This camera is connected the vision processing board, Meteor(Matrox Co., Ltd.), in the PC for vision processing. Another CCD camera with zoom lens is

connected with the image grabber board in the same PC. Vision processing uses the images through the two CCD cameras. The image data is applied for the motor control of the micro end-effector by RS 232 serial communication.

We have developed a dual illumination system that is composed of the back-light(white highly-brightness LED) and the upper-light(halogen) illumination system suitable for observing various biological cells. The XY stage on a microscope's support adjusts the position of the observed cell. The optical microscope(MEIJI TECHNO, Japan) has lens with magnification ratio 300.



A: Optical Microscope B: Holding Pipette
C: XY Stage D: Petridish
E: Zoom CCD F: 2 D.O.F. Micromanipulator
G: Injecting Pipette H: Force Sensor

Fig. 7. The proposed biological cell manipulation system.

4.2 Experiments and Discussion

To show the performance of the proposed micro end-effector, we applied it to the manipulation of the fish egg cell. Since the fish egg cell is very transparent, it can easily separate the growth procedure. Fish egg cell is composed of membrane and york. Its diameter is 700um ~ 1,000um approximately.

Fig. 8 shows procedures of the injection for fish egg cell. No.1 of the Fig. 8 shows that the pipette tip contacts with the membrane of the cell. No.2 and No.3 of the Fig. 8 show that the pipette tip penetrate the membrane. No.4 of the Fig. 8 shows that the pipette tip contacts with the york. No.5 and No.6 of the Fig. 8 show that the pipette tip penetrate the york. No.7 and No.8 show the destruction of the cell due to excessive injection force of the pipette.

We calibrated the proposed micro end-effector for its sensitivity and characteristics by using the precision scale(Satorius co., Ltd.). The scale can measure up to mN. The proposed micro end-effector was mounted to the micro stepping motor stage for linear. From calibration results, the proposed micro end-effector can sense 0.8mN force per one voltage approximately. We can find out that the force sensor of the micro end-effector has high linearity, sensitivity and good S/N ratio through the experiment.

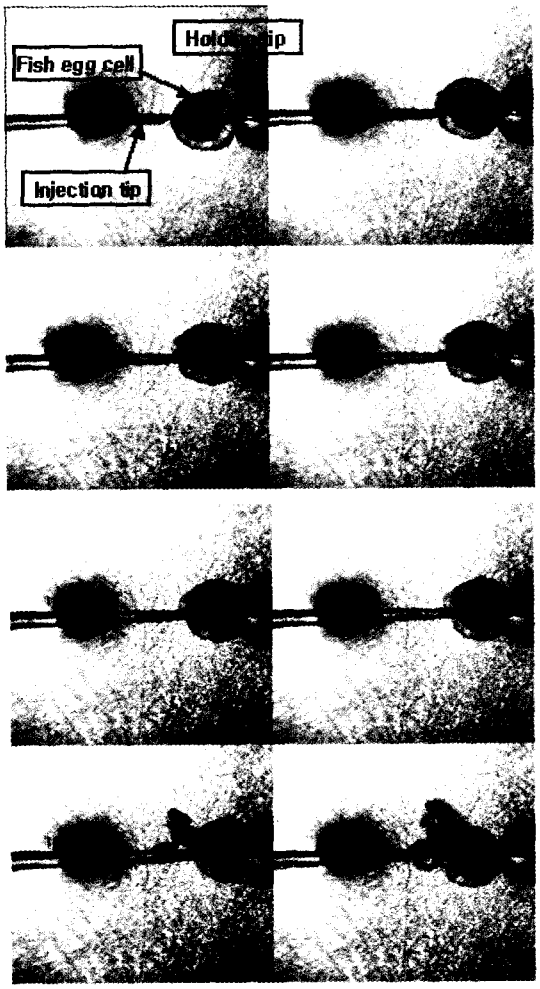
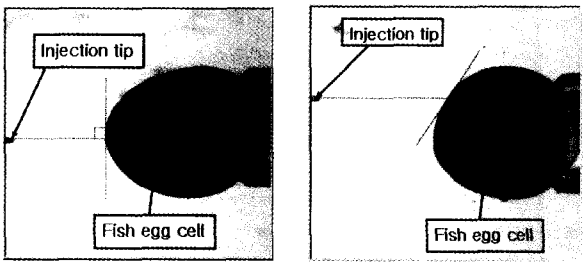


Fig. 8. Experiment of fish egg cell injection.

Fig. 9(a) shows you correct injection motion. When injected the cell, it should make tip and cell orthogonal contact each other looks like Fig. 9(a). We found out throughout the experiment; if the injection tip doesn't reach to perpendicular at the cell's tangent line, the injection tip slips at the surface of the cell's membrane. In other words, when the penetration force effect the injection tip, (or in a moment of penetration at injection tip contact the cell) Fig. 9(b) display a cause of slip situation. If the injection tip penetrated the cell like Fig. 9(b), the injection tip slide along the cell membrane and also it could make the damage at the cell membrane. Moreover, if more stronger contract force occurs, the cell is escaped form the holding tip. In this case, as the injection tip collide with a holding tip, the injection tip is wrecked.

The Fig. 10 is oscilloscope's image, when the micro

end-effector contact the cell membrane. The Fig. 10(a) is the case of contact as perpendicular direction looks like the Fig. 9(a). It could measure 3 voltage approximately. It's about 0.24mN force. The Fig. 10(b) shows slippery signal. It's non perpendicular direction as shown in Fig. 9(b). Moreover, the injection tip collided with the holding tip, also the injection tip is broken. Of course, it couldn't penetrate correctly.



(a) Normal contact mode (b) Slippery contact mode

Fig. 9. Contact mode according to the approach of the injection tip.

(a) Normal contact

(b) Slippery contact

Fig. 10. Shape of Injection forces.

We can understand the tip's penetration situation from this signal; the tip is penetrated well or, not.

We make use the vision system to prevent such a slip motion. Fig. 11 shows the vision image of the proposed micro injection system. We could observe the position of the injection tip and the contact point of the cell on the screen by vision processing. At this time, we can inspect whether the y axis values of the tip and cell are same or not. In other words, it's inspection that y axis value is same or not between injection tip and cell's injection point. If the accordance, make progress the next procedure; or not, adjust the injection tip. As a result, we can adjust the orientation of contact between the injection tip and cell membrane through the vision system.

The next procedure is measurement. In this process, vision system measure the distance between the injection tip and the fish egg cell. Measurement algorithm is simple. First, it can find the tip's coordinate, and the cell's coordinate. Finally, the value that X coordinate of the cell minus X coordinate of the tip is the distance. And the measurement result is displayed by blue circle at Fig. 11.

The distance value is sent to the motor controller by serial communication. This value is the one of X coordinate. So it is the revised motor command value for control of the injection motor. The injection motor needs 6.25 pulse for moving forward by one pixel. It is impossible to express the value of the motor pulse in a real number. Therefore, it can move one pixel by seven pulse. Since the injection distance is very short, it can ignore the small distance error.

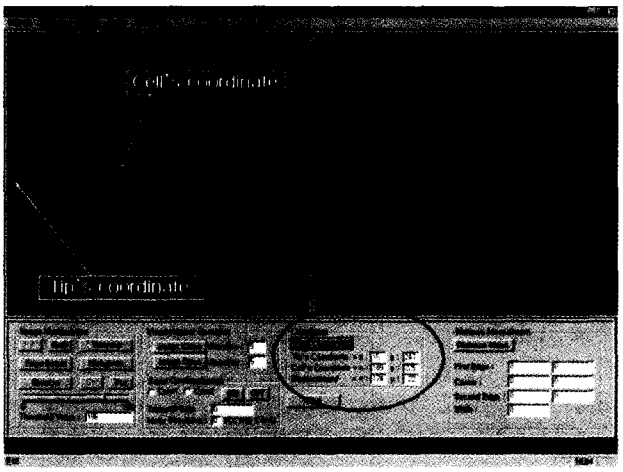


Fig. 11. Visual monitoring.

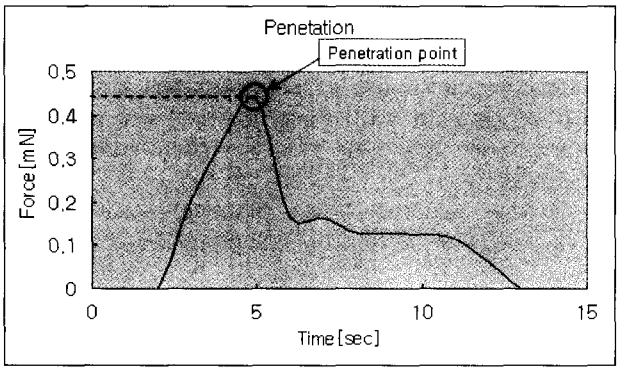


Fig. 12. Force occurred when the pipette penetrates through membrane of a cell.

The Fig. 12 displayed the state of penetration of a cell by a pipette. Force of the micro end-effector can be acquired from the data acquisition board on a PC and the force sensor. The penetration point was indicated by the

red circle line at Fig. 12. As shown in the figure, the force of penetration is about 0.45 mN.

5. Conclusions

In this paper, we proposed a new micro end-effector that is composed of strain gauge sensors, diaphragm, pipette tip, tip holder and capillary holder. The proposed micro end-effector will be used for the biological cell manipulation. Particularly, the end-effector has a sensitive force sensor inside itself. It can sense the several mili Newton force when the pipette tip contact with the membrane of the biological cell. The force sensor is a type of diaphragm. We analyzed the displacement and stress of the diaphragm according to shape and structures by using the finite element method. We designed the proper structure of the sensor diaphragm from the results of the finite element method. In this paper, the sensor diaphragm is made of SUS304. Four semiconductor type strain gauge sensors were attached on the diaphragm. We could obtained three kinds of force information such as Fz, Mx and My.

We constructed a biological cell manipulation system and tested the performance of the developed micro end-effector for fish egg cell. And we find out that when the pipette tip penetrate the membrane of the cell, it required degree of several mN force. Through a series of same experiments, we know that the force sensor of the proposed micro end-effector has good S/N(signal to noise) ratio and repeatability.

It happened to slippery, when the injection tip penetrated non perpendicular direction of cell's tangent line. There's lots of problems. First, it can not understand the physical characteristics, because it can not contact the cell membrane correctly. Second, cells injured by the injection tip. Third, the injection and holding tips are broken, because two tips collided. To prevent these problems, it must penetrate perpendicular direction of the cell membrane's tangent line. For using this method, we setup the vision processing in our penetration system. The vision system adjusted the injection tip's wrong position for penetration axis. Also vision system measure the distance between the injection tip and cell membrane; and it penetrated the cell by properly velocity profile. This method can prevent the many problems.

To improve the performance of the proposed micro end-effector, we will modify this system in near future. Particularly using the haptic devices, operator will manipulate the biological cell conveniently.

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저 자 소 개

심재홍(Jae-Hong Shim)

- Affiliation : Dept. of Control and Measurement Engineering, Professor
- Education :

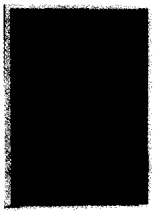
Dept. of Mechanical Engineering, Korea University, Bachelor(1985)
Dept. of Mechanical Engineering, Korea University, M.Sc. (1987)
Dept. of Automation and Design Engineering, Korea Advanced Institute of Science and Technology, Ph.D (1998)

- Careers

1987-2000 Samsung Electronics Co., Automation Research Center, Senior Researcher
2000-Present Korea Polytechnic University, Associate Professor

- Research Interests : Intelligent Control, Robot, Biological Manipulation, Sensor, Vision

Phone : +82-31-496-8248
Fax : +82-31-496-8259
E-mail : jhshim@kpu.ac.kr



조영임(Young-Im Cho)

- Affiliation : Dept. of Computer Science, Professor
- Education :

Dept. of Computer Science, Korea university, Bachelor(1988)
Dept. of Computer Science, Korea university, M.Sc. (1990)
Dept. of Computer Science, Korea university, Ph.D (1994)

- Careers

1996-Present Dept. of Computer Science, Professor
1995-1996 Samsung Electronics Co., Dept. of Multimedia, Senior Researcher
1999-2000 University of Massachusetts, at Amherst, Dept. of Computer Science, Post-doc
2000-Present International Conference on Parallel and Distributed Systems (ICPADS), PC member
2001-Present Korea Engineering Education Society, Editor
2002-Present Crevis Co., Technical Consulting Professor
2003-Present Korea Fuzzy and Intelligent System Society, Editor
2003-Present Korea Woman Information Sociery, Administrator

- Research Interests : Intelligent Multi-Agent, System, Neuro-Fuzzy system, SoftPLC

Phone : +82-31-656-9743
Fax : +82-31-656-9743
E-mail : yicho@ptuniv.ac.kr

김종형(Jong-Hyeong Kim)

- Affiliation: School of Mechanical Design and Automation Engineering, Professor
- Education:

Dept. of Mechanical Design Engineering, Seoul National University, Bachelor (1984)
Dept. of Production Engineering, Korea Advanced Institute of Science and Technology, M.Sc. (1989)
Dept. of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Ph.D (1995)

- Careers

1984-1987 LG Electronics Co., Research Center, Researcher
1995-2002 Samsung Electronics Co., Mechatronics Center, General Manager
2002-Present Seoul National University of Technology Associate Professor

- Research Interests : Machine Vision, Intelligent Control, Robotics

Phone : +82-2-970-6357
Fax : +82-2-974-8270
E-mail : johnkim@snut.ac.kr