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Development of a Compact Vision System for "Automated Nuclear Transplantation Project"

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Abstract— **This paper describes research and development of a compact vision system for real-time cell detection and manipulation in a capillary in order to apply on the "Automated Nuclear Transplantation" project which will achieve sort of cell manipulation tasks such as positioning, cutting, sizing, and so on, automatically between different interconnected modules. Here, we propose a vision system boarded on a PDMS based silicon chip, which can be utilized in a complex network for continuous monitoring of mammalian egg and donor cells of sizes in the range of 10 to 100 micron. The developed prototype has sufficient resolution and is accompanied with a robust detection method for cell-based microfluidic applications.**

Index Terms — PDMS, Micro Fabrication, Optic Systems

I. Introduction

There is a noticeable growth in micro system technology for biomedical related products such as biosensor, biochip and microfluidic devices. Recent micro-fabrication techniques on microfluidics structures have significantly enhanced the manipulation of cells for biomedical applications [1-5]. Especially, soft lithography has been successfully applied to produce microfluidic channel structure in which biological particles can be observed and manipulated. One of the most common elastomer used in soft lithography for biological applications is the PDMS (Polydimethylsiloxane), which is non-toxic to living cells and impermeable to water. Furthermore, cured PDMS has low surface energy preventing specimens in the PDMS microfluidic channel from interacting with it. Besides, optical properties of PDMS (transparent down to 300nm of wavelength) have key functionality for several kinds of cell monitoring MEMS applications [6-8]. Therefore, PDMS has remarkable advantages for prototyping and fabricating a variety of microfluidic mechanisms, which have been used in lab-on-a-chips, existed for almost a decade.

Lab-on-a-chip (LOC) devices are attractive for biomedical application because of their portability, reduced size, disposable structures. Their inexpensive composition made them a potential candidate for large scale mass production. It covers not only the material phenomena but also the technology for manipulating and controlling the components as micron size particles in micron size artificial capillary.

Microfluidic devices are one of the important research areas for driving infinitesimal amount of solution in a micro channel. Cell-based microfluidics has become a versatile tool for biological research. There is a wide range of methods by which quantitative biological information such as staining intensity and label specificity; cell number, size, and volume; and distribution within a field of view may be extracted from microscopic images during biological particle transfer [9-11]. For example, as method of controlling particles during flow, several methods employ different strategies to define location or size of a single cell. Image processing methods may provide an efficient solution to manage and navigate particles in micro-channels. Through the use of special algorithms, images may be automatically processed on basis of user-defined criteria. Such automation can greatly aid image processing especially where the manual data extraction would be impossible, tedious or error prone [12]. Recently automated microscopy systems have been studied with different aspects [13].

The volume of current microscopic vision systems is an obstacle for their use in LOC devices. For typical lab-on-a-chip devices, vision systems are only required during development phase, and the final detection is done by hand-held electronic reading devices rather than optical vision systems. The miniaturization of vision systems and their integration on LOC devices promise new sensing methods accompanied by portable monitoring technologies. On the other hand, conventional optical system such as microscope objective does not bring adequate solution to integrate on a chip because of their big sizes. They also need additional components to attach on a chip, which is also increase volume of the chip.

In this paper, we describe our vision system, which can be integrated into every module of desktop bio-plants. It provides sufficient magnification and, is driven by a motion based imaging algorithm to detect single cell in a capillary. The integration technique of the whole system into a PDMS chip is reported and the procedure is listed step by step. By using this new method, the prototype system can efficiently embedded on a chip and acquire fine quality image. The resolving power under the desired field of view dictated by

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pre-defined requirements such as minimum image extraction limits are depicted in the experimental results section. It also includes the performance of the complete system for cell sizing and accurate positioning. The advantages of complete system are briefly summarized in conclusion section.

II. Design Criteria

State-of-the-art desktop bio-plants, which will perform several cell manipulation tasks such as positioning, cutting, sizing, etc., on different interconnected modules have increasingly been in demand for nuclear transplantation operations in biomedical science. Manual cloning operations, such as extraction of the original nucleus and insertion of the donor one, take a long time and success rates are quite low. Therefore, more effective and less time-consuming techniques are required, and desktop bio-plants are accepted as good candidates.

We aim to design a compact, economical and task-adaptable sensing and cell-actuation system which we call as "Automated Nuclear Transplantation Using Micro Robotics." The system includes micro-channel, micro-optics and micro-wells on a chip with suitable sensors and actuators. The ultimate goals of the desktop bio-plant project are the miniaturization of the nuclear transplantation technology to provide a fully automated and high-throughput system.

A key requirement in the successful implementation of such a system is the real-time cell detection, tracking, sizing and manipulation, which are the essential parts of the micro-robotic applications. The current embedded lab-on-a-chip sensor utilizing light scattering, electrical resistance, and sound method technologies, are not sufficient to extract desired information about a cell through the complex steps of nuclear transplantation. For example, in the cell-fusing phase of mammalian cloning process, two cells (donor and egg cell) are brought into very close contact, and aligned via dielectrophoresis after which an alternating voltage is applied for a brief period to complete the fusion. Although the outer cell membranes are fused with the application of the alternating voltage, without observing the evolution of the shape of the cell during the entire fusion process, one cannot be sure of the formation of the new cell, which is a product of the mingling of cytoplasmic contents including organelles and nuclei of both cells. A fusion process is successful when the new cell has a near circular shape as illustrated in Figure 1. The existing methods lack the ability to correctly detect the shape in a non-destructive way. Thus, image analysis methods are proposed as an alternative to the existing methods, and it is crucial to miniaturize the image analysis system to accommodate in the desktop bio-plant.

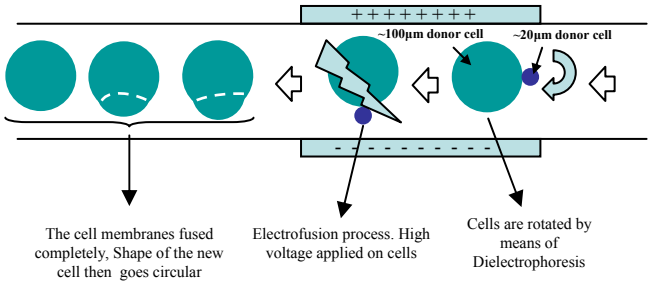

Figure 1. Demonstrates cell fusion process in a micro channel. Each step needs image information to produce the desired results.

The extraction of the desired information accurately during the process requires image collection of both the donor and the egg cell followed by a number of processing steps: thresholding, filtering, contour processing, background subtractions, removal of optical artifacts and other transformations.

Our up-most concern is to make a compact system that is able to monitor a micro-channel and to detect the cells of size in the range of 9.5µm to 100µm and implement on a chip without spoiling the compactness of LOC. We have designed and prototyped a miniaturized imaging system which provides a good image quality allowing extraction of desired information of the egg cell and donor cell from the acquired images. However, the most of LOC devices have disposable parts, unlike micro channels, the prototype vision system do not engage with any disposable components due to its expensive structure.

III. Compact Vision System

A. Design of the Optical System

The proposed compact vision system employs two achromatic doublet lenses with convex crown surface facing each other. This structure has considerably higher optical performance and also gives us admirable image quality while having low spherical aberration.

A set of paraxial calculations, where some functional parameters such as the conjugate distance and magnification of a vision system are taken into account, was performed. The lens system was set up for the desired magnification, which is calculated by considering the required field of view and CCD size. Pre-defined field of view was set as 1.00 mm for dexterous manipulation and analysis of the both donor and the egg cell cells. The image height was taken 1.9mm as a lateral size of the CCD. Then the magnification was calculated using [14]

$$M = \frac{H_i}{H_o} \qquad (1)$$

where H_i and H_o are the heights of image and object, respectively. In our system, taking Hi=1.9mm and Ho=1.0mm yields M=1.9.

$$f_{ab} = \frac{f_a f_b}{f_a + f_b - d} \quad (2)$$

where d is the distance between two achromat lenses, and f_a and f_b are the focal lengths of the two lenses in the system, we calculated the EFL (Effective Focal Length) of the lens-combination. In our scheme, f_a = 10mm, f_b =10mm and d is taken as 0.5mm resulting in EFL = 5.12mm. Substituting the calculated EFL and M in Gaussian equations (3) and (4), yields the positions of the object and image as

$$s = f_{ab}(1 - M) = 5.12(1 + 1.9) = 14.84mm \quad (3)$$

$$s' = f_{ab}(\frac{1}{M} - 1) = 5.12(\frac{1}{-1.9} - 1) = -5.64mm \quad (4)$$

where s and s' indicate the distance of the image and object to the lens system, respectively.

B. Monitoring System

The detection optics detailed above was combined with 0.34 MPixels CCD sensor and bended 90° degree with the aid mirror in order to avoid unwanted horizontal growth on the size and to provide compactness as well. Objective and CCD distance were adjusted considering the given field of view (1mm) under the desired magnification (M: 1.9X).

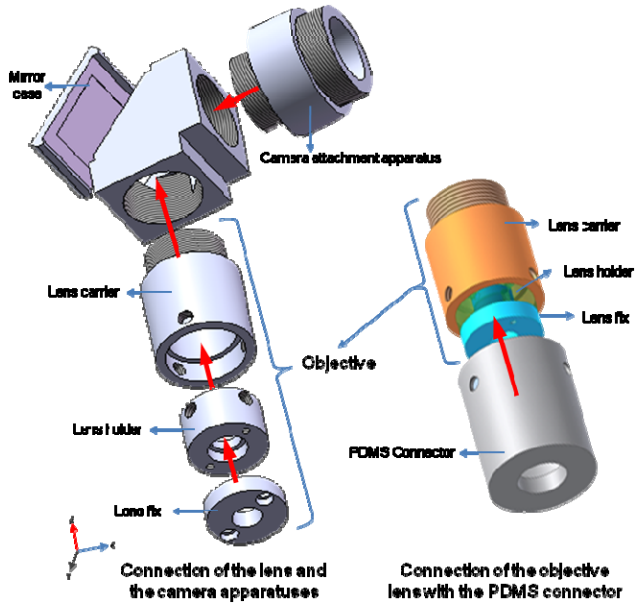

Figure 2. The compact vision system structure.

To focus the camera, the objective must be moved near to or further from the sample. Every focusing apparatus for monitoring system is of the single shaft coarse movement type in which a coarse adjustment knob and fine adjustment knobs are disposed coaxially with each other. The coarse adjustment knob is also utilized as a PDMS connector. When the coarse adjustment knob is rotated, whole system can be vertically moved relatively greatly and when the fine adjustment knobs are rotated, the stage can be horizontally finely moved. A coarse and fine adjustment focusing apparatus of the entire system and fine adjustment knobs are shown in Figure 2.

As described above, when quick focusing is necessary, the coarse adjustment knob is rotated, and when accurate focusing is necessary, the fine adjustment knobs are rotated, whereby appropriate focusing is made possible.

C. Illumination System

Besides the objective and the camera systems, the illumination system is also a critical part of the experiments. If it is well adjusted, it allows objects to be seen with a good sharpness at the CCD sensor, otherwise scattering light will confuse the fine details of the image. Illumination system was shown in Figure 3.

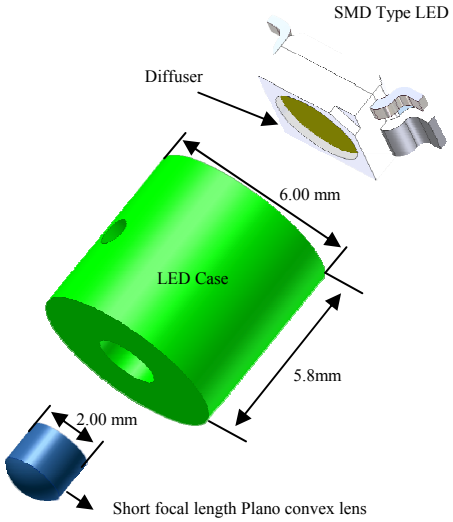

Figure 3. **Illumination System.**

For illumination, we used light radiated by a "Toyoda-Gosei" SMD type LED (Type No: E1SM1-0W0M5-05) which has 3.5 X 6.0 X 1.9 (mm) package size, and emits highly intensive white light. Spectrum value of the light varies between 400 nm to 700 nm. We placed a frosted diffuser in contact with the LED to have a uniformly diffused illumination throughout the cell manipulation and detection region. Right after the frosted filter, we placed a plano-convex lens (2 mm) as a collimator to illuminate the micro-channel. The oblique rays which exit the source are transmitted through the filter where the intensity level is decreased depending on the grid density of the frosted filter. The level of incident light was smeared equally over the front face of the filter and it yields an almost uniform light. Thereby, the lens collimates the light from the filter. This gives better illumination conditions and avoids problems due to short-focus lens adjustment near source of light.

D. PDMS Integration

The PDMS preparation and the integration of imaging system are depicted in Figure 4. First, an initial PDMS layer (depicted as first layer in Figure 4) including micro channels was prepared. The inlet and outlet points were connected to silicon tubes before mounting. The micro-channels were patterned on negative mold master by pouring PDMS mixture, which was deggased in vacuum conditions. Cured prosthesis, 20 min under 100°C in an oven, was peeled from mold and treated by air plasma using expanded plasma cleaner for 1 minute with slide glass which was rinsed in the ethanol. This constitutes the first layer of the whole chip and slide glass is bounded. Each layer is a complimentary part of each other, and works like one layer until reaching the top level.

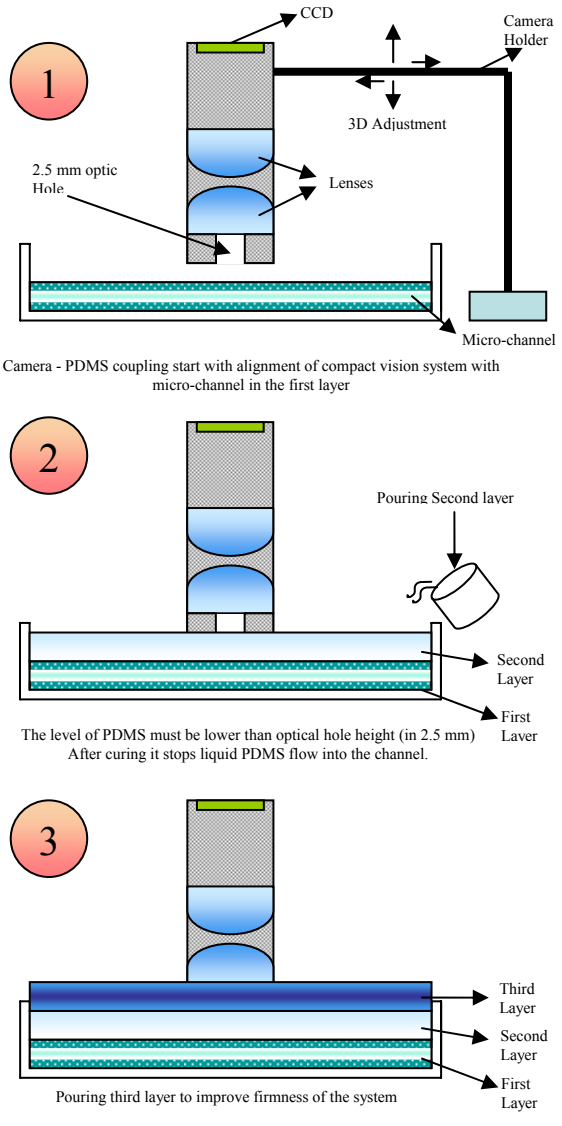

Figure 4. The Compact vision system integration in PDMS chip.

After the each of the optical component was aligned properly and fine adjustments of the connectors were done, the vision system was integrated to the microfluidic chip in three essential steps: (i) Precise alignment of optical axis about micro-channel by the aid of external micro-holder in x,y,z directions, (ii) The second layer PDMS is decanted between microfluidics and lens connector until it exceeds the level of connector about 1mm. This layer blocks the liquid PDMS flooding into objective hole and replaces the air. (iii) Third layer PDMS is poured into silicon structure to jam vision system and not to shift around. The chip and the integrated vision system are shown in Figure 5.

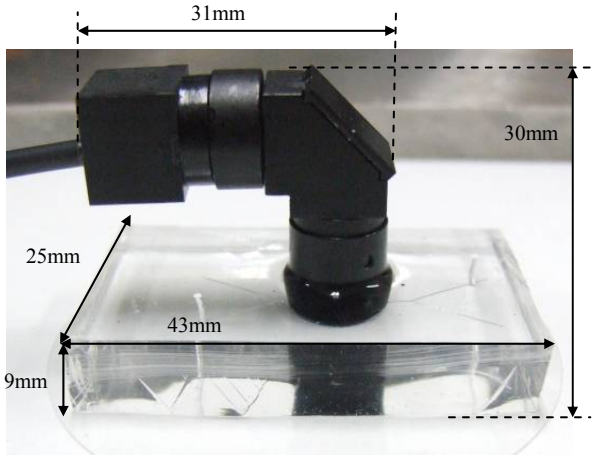

Figure 4. The Compact Vision system coupled with PDMS chip.

IV. EXPERIMENTAL RESULTS

Automatic detection and tracking of a particle are critical functions to accomplish well-control manipulation. Every real time detection algorithm must identify tracked object according to environmental parameters. In our system, illumination variability, depth of field, cell deformation may change detection task accuracy in some cases such as out of fine range focusing. The dynamic background subtraction algorithm was employed through detection phase to eliminate redundant artifacts and to surpass optic based aberrations. Due to dynamic environment in micro channel, and bubbles which have similar shape as test particles, the total system efficiency was decreased somewhat. Additionally, the disadvantage of using fixed objective caused lack of resolution of specimen and led to detection difficulties. Even though the circumstances were so, the tracking algorithm was successfully implemented to various tasks.

Using the integrated system, we performed a number of experiments to test the efficiency and feasibility of our integrated systems.

In the first Experiment (Figure 6), we show the image captured during the experiments for a cell of estimated size 26micrometer. The position of the cell in the channel could be observed. In doing this, we used minimum enclosing

circulation method, which is run in accordance with the information of contours processed image, on the visual data and results of the position records updated simultaneously. At the final step, a continuous control operation was done by a simple P type controller fed by incoming cell data.

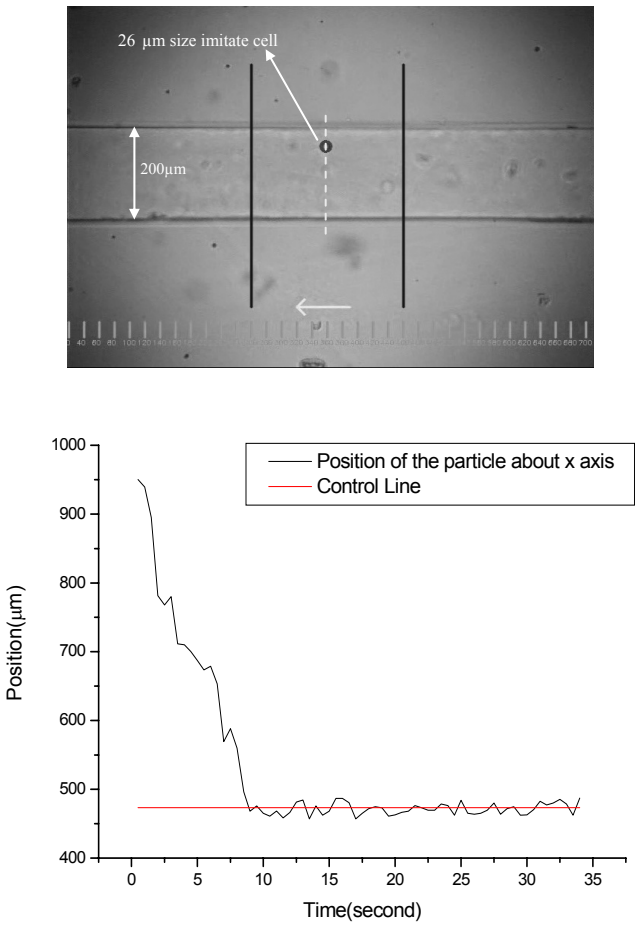

Figure 5. External micro-pumping device (ISMACTEC high precision tubing pump) was operated to manipulate flow speed and direction in the capillary to make localization through desired area in terms of the visual feedback. The identified cell region was used as a control parameter in order to provide coordination between pump and tracking software.

The Second experiment was performed to test the effect of the frosted filter in the captured images. In Figure 7, we show the images taken with and without the frosted diffuser. As it is seen, without the diffuser there is a bright spot in the detected image due to the non-uniform illumination. This bright spot is significantly suppressed with the introduction of the diffuser. The diffuser allowed a uniform illumination over the monitoring region while allowing an enhanced contrast factor

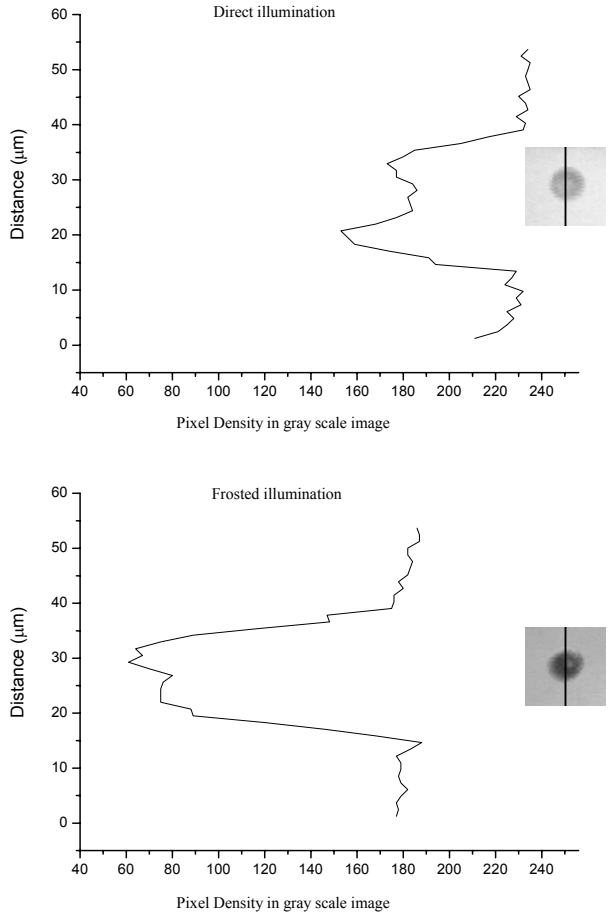

Figure 6. Single particle in 26µm was determined by their optical density. Graphs show the effect of frosted filter on resolution.

Last experiment was performed to evaluate the system in finding the size of the particles. The experiment was repeated for particles of different sizes and good agreement was observed between the measured values and the values in the data sheet provided by the supplier. The results for the particles were taken from the batch of particles with average size of 11 micrometer are shown in Figure 8. Experiments were performed as follows: From the batch of 10micrometer particles, we randomly sampled 10. For each particle we performed 100 measurements. From the captured images of the moving particles we calculated the average size and the standard deviations. Thus, each point in the figure is the mean size of the measured particle together with the standard deviation. As it is seen from the figure, the measured values lie within the range of possible particle size given in data sheet by the supplier who claims a 18% size uniformity for the 11 micrometer batch.

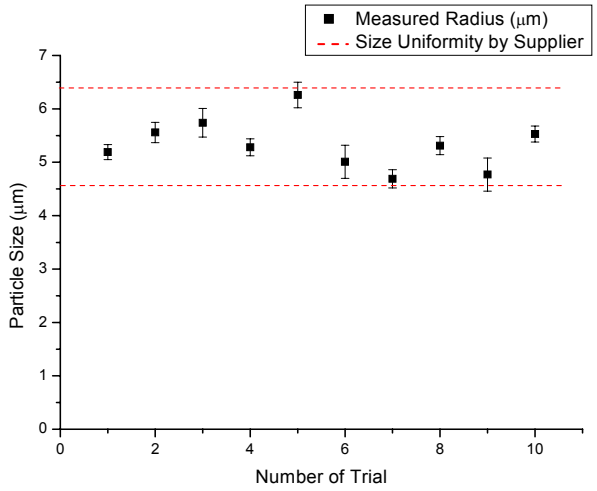

Figure 7. Each particle size was determined after 100 measurements and then average value for each was put on the graph. Standart deviation values after 100 measurements were placed on each. Measurements were sampled within the red lines, which show the real size uniformity given by supplier. While our measurements were done by our imaging algorithm, for provided suppilers values, measurements was taken by N.I.S.T. traceable test standard.

V. Conclusion and Future Work

Each module of the Desktop-bioplant carries out a specific process such as cutting, sorting, filtering, positioning, and fusing, all of which are required in nuclear transplantation process. Instant observation and examination of an egg-cell or a donor cell have paramount importance for these operations, and are needed in essential steps of nuclear transplantation (as cell fusion). Our experiments show that the prototype vision system and practical solution of PDMS integration of that could be done with care and, design requirements such as resolution range could be achieved in a short distance while maintaining compactness.

In this paper, fundamental concepts and some application of miniaturized vision system have been described with different aspects. The optic and illumination system design were briefly explained, and resolving power has been shown through the test on 10 micron particles. For the illumination system, we emphasized that proximity of interference filter play a crucial role in this experiment.

Detection method developed for microfluidic systems is a simple and robust technique. The algorithm was used for different tasks to find out the performance of the system. Through the sizing test, it has potential to resolve and to identify the cells in the channel. The information extracted from images was used to control the motion of the particles in the channel. Our system can be easily incorporated into microfluidics and utilized for the monitoring of process ongoing in the channel.

References

[1] T. Vilkner, D. Janasek and A. Manz, "Micro total analysis systems. Recent developments", Anal. Chem. 76, 3373-3386 (2004).

[2] . A. Unger, H. P. Chou, T. Thorsen, A. Scherer and S. R. Quake, "Monolithic microfabricated valves and pumps by multilayer soft lithography", Science 288, 113-116 (2000).

[3] E. W. H. Jager, O. Inganäs and I. Lundström, "Microrobots for micrometer-size objects in aqueous media: potential tools for single-cell manipulation", Science 288, 2335-2338 (2000).

[4] Li, G.P., "Micro/nano technology: a solution for next generation multi-function integrated systems," Bipolar/BiCMOS Circuits and Technology, pp. 201-208, 2004

[5] Ramesham, R.; Ghaffarian, R., "Challenges in interconnection and packaging of microelectromechanical systems (MEMS) Electronic," Components and Technology Conference 50th , pp. 666-675, 2000

[6] YC Lee, BA Parviz, JA Chiou, S Chen, "Packaging for microelectromechanical and nanoelectromechanical systems," IEEE Trans. on Advanced Packaging, vol. 26, no. 3, pp 217-226, 2003

[7] Y. Cui, Q. Wei, H. Park C. M. Lieber, "Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species", Science 293, 1289-1292 (2001).

[8] M. A. Schwarz and P. C. Hauser, "Recent developments in detection methods for microfabricated analytical devices", Lab Chip 1, 1-6 (2001).

[9] Scho¨nholzer, F., D. Hahn, B. Zarda, and J. Zeyer. 2002. Automated image analysis and in situ hybridization as tools to study bacterial populations in food resources, gut and cast of Lubricus terrestris L. J. Microbiol. Methods 48:53–68.

[10] Elfwing, A., Y. LeMarc, J. Baranyi, and A. Ballagi. 2004. Observing growth and division of large numbers of individual bacteria by image analysis. Appl. Environ. Microbiol. 70:675–678.

[11] Blackburn, N., A. Hagstro¨m, J. Wikner, R. Cuadros-Hansson, and P. K. Bjørnsen. 1998. Rapid determination of bacterial abundance, biovolume, morphology, and growth by neural network-based image analysis. Appl. Environ. Microbiol. 64:3246–3255.

[12] Daims, H., N. B. Ramsing, K.-H. Schleifer, and M. Wagner. 2001. Cultivation- independent, semiautomatic determination of absolute bacterial cell numbers in environmental samples by fluorescence in situ hybridization. Appl. Environ. Microbiol. 67:5810–5818.

[13] Price, J. H., A. Goodacre, K. Hahn, L. Hodgson, E. A. Hunter, S. Krajewski, R. F. Murphy, A. Rabinovich, J. C. Reed, and S. Heynen. 2002. Advances in molecular labeling, high throughput imaging and machine intelligence portend powerful functional cellular biochemistry tools. J. Cell. Biochem. Suppl. 39:194–210.

[14] Sidney F. Ray, "Applied Photographic Optics", Focal press, 3rd ed., ISBN 0240515404, 2002