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A micro flow cell cytometry based on MEMS technologies using silicon and optical fibers

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In this paper, we tested multi-mode optical fibers to select a suitable fiber for effective flow of cell cytometry. In order to align micro nozzle and multi-mode optical fibers, a guide channel was fabricated by silicon wafer etching with MEMS (Micro Electro-Mechanical System) technologies. The fabricated system is advantageous due to its low cost and simplicity in construction. It is possible because multi-mode optical fibers replace many optical lenses and expensive equipment. As a result of the flow cell cytometry using multi-mode optical fibers for both input and output, it is easy to align and we can reduce power consumption. The sensitivity of the micro flow cell cytometry is much better than other cytometries. The output voltage was as high as 300 mV. We injected various cells through the designed and fabricated flow cell cytometry, and we were able to detect cells. Every cell has its own cellulose and wall which cause different light permeability; therefore, we could get different voltage characteristics according to different cells. From the experimental results, we were able to count the number of cells and differentiate the relative size of the injected cells; therefore, we can use the micro flow cell cytometry for analyzing cells [1, 2].

1. Introduction

The micro flow cell cytometry analyses equipment that measures the cells with an optical signal obtained from each cell by applying a laser light to the cell. In biology, a cell phenomenon is measured in real time by categories based on classification by biological components and structures. Therefore, we expect that this micro flow cell cytometry can contribute to the development of biological engineering and clinical testings. Micro flow cell cytometry can be practically used in the diagnoses of particular diseases and biological symptoms and in *in vitro* diagnostics when it gets smaller. The micro flow cell cytometry, however is not easy to do research using general cells analyzing equipment since it is an expensive and complicated method to use for research. Therefore, we are fabricating a micro flow cell cytometry using optical science and MEMS technology.

Further more, we could make the renovated micro flow cell cytometry smaller by using optical fibers This helped us to reduce the cost and simplify the structure of the flow cell cytometry. The optical fiber replaced many optical lenses and other high-cost equipment. This experiment is about fabricating the micro flow cell cytometry that can analyze cells using optical science and MEMS technology. We were able to make a low cost and simple cytometry by using optical fibers instead of high cost optical lenses and other complicated optical devices. Also, we fabricated the flow cell parts using MEMS technology solving the micro nozzle and optical fiber alignment problems. The operating principle of the micro flow cell cytometry is as follows: As we injected cells into the micro nozzle. We focused a laser light on the micro nozzle sensors using an optical fiber. We were able to get an optical signal from the micro nozzle. The optical signals were transferred to the optical fiber. The transferred optical fibers were then amplified by a photo diode and PMT (Photo Multiplier Tube). The transferred optical signal converted analog signals to digital (ADC: Analog to Digital Converter) using the fabricated circuits and collects information using a computer program. The output signals were measured and analyzed. It sensed the flow cells using an optical fiber. Also, we designed and fabricated the cell counters to count the number of cells and microbics in a fixed quantity solution. For programming, Visual Basic was adapted. The micro flow cell cytometry proved possible as a cell analyzer as a result of test injecting leukocyte of Mouse T cells (EL-4, 6×105/ml), human erythrocyte and leukocyte cells and the inner cells of blood vessels to the system [3, 4].

2. Fabrication

In order to align optical fibers and the micro nozzle, the guide channel is fabricated of (100) silicon wafer with

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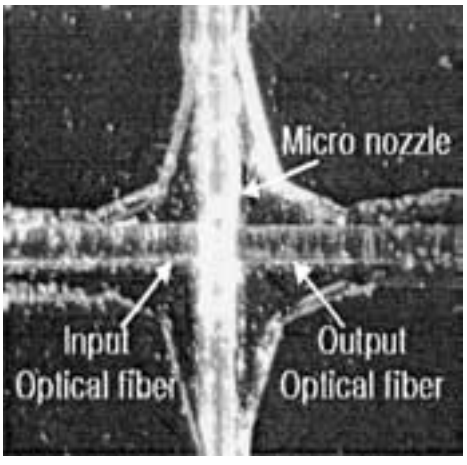

Figure 1 Photograph of fabricated guide channel.

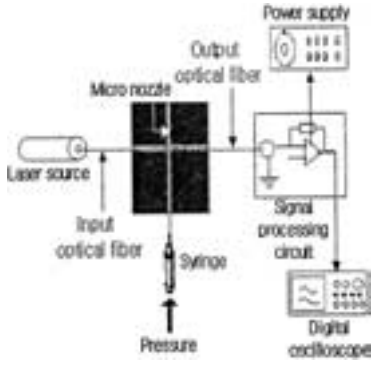

Figure 2 Schematic diagram of fabricated system.

MEMS technology. The Si wafer was etched by TMAH (20.0 wt%, 90°C, etch rate: 0.97 μm/min). To protect from outside impact and misalign between the micro nozzle and optical fibers, the anodic bonding (1000 V DC, 400°C) was executed for Pyrex glass and Si wafer which guide channel is fabricated on.

The aligned photograph of optical fibers and the micro nozzle on the guide channel is shown in Fig. 1. In order to detect a small optical signal through the output optical fiber, we designed a signal processing circuit with a photo diode (S2386-8K, TO-8 type). We made the cell countable circuit with a comparator (LM311).

The circuit for amplification and cell counting is used a data acquisition system (DaqBook/100) and a personal computer to process the output signal from an electrical circuit. The digital oscilloscope (LeCroy LC534A) is used to measure effectively the changes of a light quantity. Then we made a program using Visual Basic to observe the output of the cell counter.

Fig. 2 shows a schematic diagram of the micro flow cell cytometry system. As a result of injecting Mouse T cells (EL-4, 6 × 105/ml) 0.3 ml in the fabricated system. The result the output of the cell counter, it was able to detector 22,917 cells for 8 min and 34 s. The average counting speed was 45 cells/sec.

3. Experiment and results

A test was performed by pouring mouse T cells (EL-4, 6 × 105 cells/ml), Human T cells (2 × 106 cells/ml),

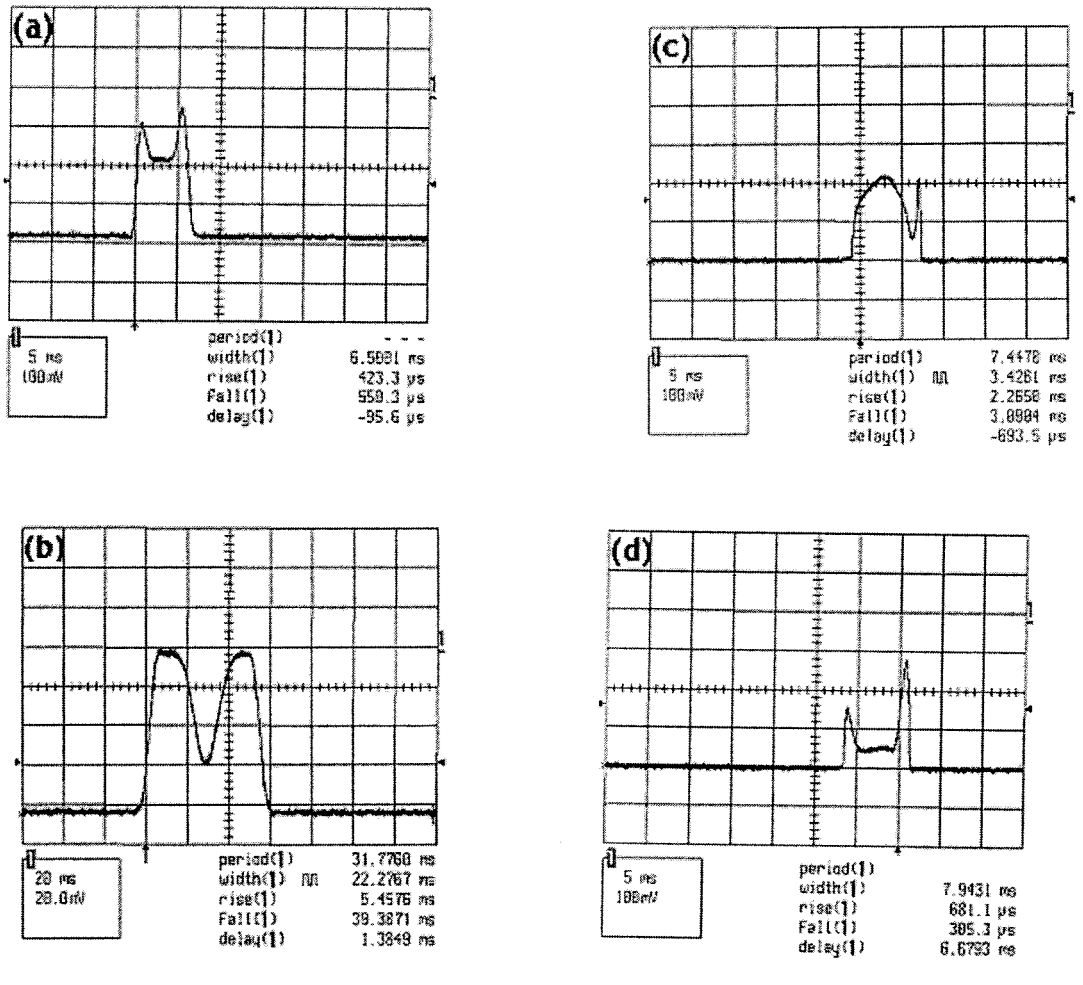

Figure 3 Voltage characteristics of cells. (a) Mouse T cell. (b) Human T cell. (c) Human huvec. (d) Human red blood cell.

Human huvec (Phenol Red stain) and Paraformaldehyde Huvec into the system. The characteristics of the laser diode module uses stabilized optical output power of 25 mW, low power consumption, a high power laser beam, a 3 element glass lens, single supply voltage (5 V DC), over current protection, typical wave length of 658 nm, 20 mW, and a wire length of 30 cm or custom. The cells were injected into the system at a constant speed of 0.58 μl/sec.

The input and output in Fig. 3a were measured using multi-mode optical fiber and it shows the voltage character of the mouse T cell. Fig. 3b is a Human cell and the measurements using an optical fiber at input and output showed a voltage curve of about 80 mV. Fig. 3c and d show the output waves of Paraformaldehyde Huvec and erythrocyte respectively, and multi-mode optical fibers were used at both the input and output. We found the output characters of cells were different from cell to cell. That can be attributed to the different light transmissivity of each cell because of differences in cytoplasm and cell membrane. Also, when cells were injected into the system at a constant speed, we found the curve width to be proportionate to the size of the cell. Figs. 4 and 5 are the voltage curves that measure the injection of human T cells and we can compare the size of the cells. The cell in Fig. 4 is about 1.5 times larger than the cell in Fig. 5.

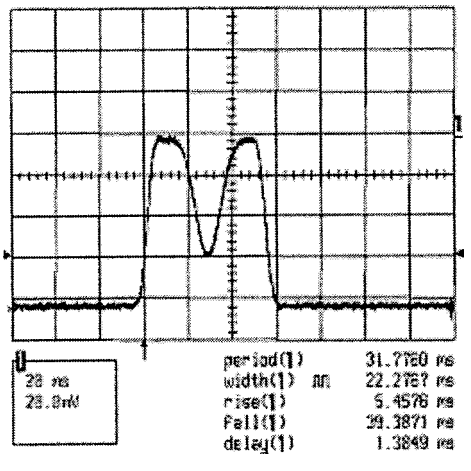

Figure 4 Voltage characteristic of Human T small cells.

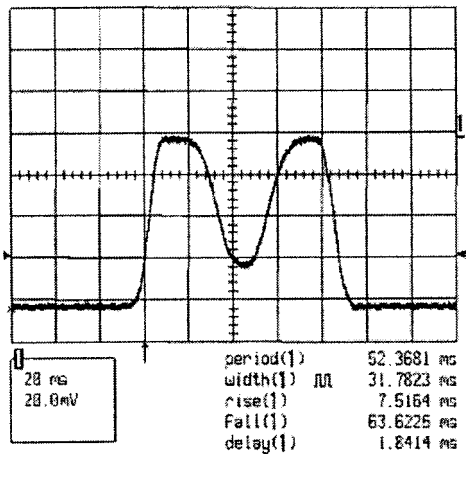

Figure 5 Voltage characteristic of Human T large cells.

4. Conclusion

We can see the flow of cells after injecting various cells in the system. Also, we measured an average sensing speed of 45 cells/sec as the result of experiments with the cell counter that can count the number of cells in the solution. The speed is more than two times faster than those of micro cell analyzers developed recently [5]. In terms of fabricating costs, our micro flow cell cytometry replaces the high cost and the complicated optical lenses, solves alignment problems, and reduces costs by using multi-mode optical fiber at input and output. Thus, our micro flow cell cytometry producing output signal even with low output light sources [6]. We fabricated micro flow cell cytometry and analyzed cells that were injected into the system. As a result, we found the correlation of the size of cells. Therefore, we have no doubt that this micro flow cell cytometry can contribute to the development of biological engineering and clinical testing and can be practically used in the diagnoses of particular diseases, biological symptoms and *in vitro* diagnostics [7, 8].

Acknowledgments

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