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Compact disk (CD)-shaped device for single cell isolation and PCR of a specific gene in the isolated cell

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Abstract For immediate discrimination among isolated cells we propose a novel device and technique for isolation of cells and sequential detection of specific gene(s) within them by polymerase chain reaction (PCR). In this study, we isolated *Salmonella enterica* cells and detected the S*almonella*-specific *invA* gene from isolated cells by PCR on a compact disk (CD)-shaped device. This device enabled liquid flow by centrifugal force without a micro pump, and was fabricated from silicon wafer and glass to avoid evaporation of a small amount of reagent. One device has 24 microchannels, and 313 microchambers integrated on each microchannel. One microliter of PCR mixture containing cells was separated into microchambers on the device at 5000 rpm for 30 s. Each microchamber contained approximately 1.5 nL PCR mixture. A Poisson distribution of *S. enterica* cells was observed for different densities of cell suspension. At 200 cells μL^{-1} of *S. enterica* or less, isolated single cells could be determined on the device by amplification of DNA of the *invA* gene; at 400 cells μL^{-1}, chambers containing no, one, two, or three cells could be determined on the device. Selective detection of *S. enterica* was achieved by PCR from a mixture of *S. enterica* and *Esherichia coli* on the CD-shaped device.

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Introduction

The development of miniaturized micro systems for chemical and biochemical analysis based on the concept of the so-called "lab-on-a-chip" is, because of their desirable characteristics, currently attracting major interest. Such micro systems enable the miniaturization of conventional bench chemical systems to a few square centimeters in size. Advantages include reduced reagent consumption, space requirements, and analysis time [1, 2]. Micro chemical devices have several remarkable advantages, for example short diffusion distances, low Reynolds numbers, high interface-to-volume ratios, and small heat capacities. By exploiting these advantages, new methods utilizing integrated miniaturized chemistry have led to the creation of extremely efficient devices to carry out chemical tasks that could not be realized on a normal scale. Such devices are expected to be applied to various fields, for example analytical, biological, clinical, medical, and industrial chemistry. The handling of a small amount of reagent has

enabled the manipulation of a single cell on micro chips [3–6]. Furthermore, labs-on-a-chip cut costs significantly because of the handling of a small amount of reagent.

Large variation in gene expression and behavior has recently been observed for genetically identical cells [7, 8]. However, it is impossible to predict the behavior of individual cells from the averaged information of many cells, because intrinsic phenomena are hidden behind averages. By isolating a single cell from a large number of cells, understanding of an individual single cell should be clearly established. In single-cell analysis, various methods to control cell position exactly have been developed with easy feeding of reagent [9–16].

We have developed a novel single cell isolation method on a CD-shaped device with an original fluidic pattern. In the device, cells were principally isolated by centrifugal flow in a Poisson distribution, irrespective of cell size [17]. The device was made of polydimethylsiloxane (PDMS) and a glass plate. Details of the CD-shaped device are given in Electronic Supplementary Material Figs. S1 and S2. A microchannel was designed in a zig-zag shape from the center to the periphery on a CD-shaped device to enable liquid flow by centrifugal force without any micro pump. The zig-zag shape is useful for applying the centrifugal force in the same direction constantly. The dimensions of the microchamber are 300 μm (width)×200 μm (height)× 46 μm (depth). The width of each channel is 100 μm. Isolation of *E. coli* and Jurkat cells on this CD-shaped device in only 30 s has already been reported [17].

Since polymerase chain reaction (PCR) was first described in 1985 [18], it has become an essential tool in the field of genetic analysis, providing a method of amplifying DNA sequences of interest. The speed of the conventional technique needs to be improved, because it is still time-consuming, and the reagents are expensive with substantial volumes needed for processing of samples between pre-treatment, amplification, and analysis steps. High-throughput PCR strategies have focused on smaller reaction volumes to reduce experiment cost and time. Miniaturization of PCR has the potential to drastically reduce amplification time and reagent consumption while simultaneously improving the efficiency of the reaction [19]. Miniaturized micro plates based on high-density arrays with wells etched in a plain substrate are the basis for nanoliter-scale [20–23] or picoliter-scale [24] PCR. To understand the life of cells by the minute, a technique that can isolate a single cell and detect a specific gene from that isolated cell is indispensable. However, there is no device that combines isolation and detection of a specific gene for multiple isolated cells.

In this paper, to determine a specific gene for isolated cells, we propose a novel device and technique to isolate cells and sequentially detect a cell-specific gene by PCR.

For single cell isolation, the micro fluidic design was based on the method we have already reported [17]. The base material was changed from PDMS to silicon wafer to enable PCR on our device. DNA amplification was run in the same chamber of the same device after single cell isolation. The unique feature of PCR on this device was that the PCR sample was completely separated by air in the microchannel. On common micro fluidic devices for PCR, generation of micro bubbles in the channel by heat is a serious problem that disturbs the solution flow. Our CD-shaped device was used to overcome the problem. In this study, we examined *Salmonella* as a model cell to demonstrate the identification of an isolated cell by detection of a specific *Salmonella* gene, *invA*, by PCR amplification on our CD-shaped device.

Experimental

CD-shaped device fabrication

The microchannel of the CD-shaped device was designed in a zig-zag shape in accordance with to our previously reported pattern [17]. Details of the CD-shaped device, the microchannel, and the microchambers are given in Electronic Supplementary Material Figs. S1 and S2. Twenty four microchannels are arranged on a CD-shaped device, each of which has 313 microchambers. Many U-shaped microchambers are arrayed on the outer side of the microchannel. The width of each channel is 100 μm. The dimensions of the microchamber are 300 μm (width)× 200 μm (height)×46 μm (depth), the pitch between microchambers is approximately 200 μm, to discriminate the fluorescent image of PCR products, and the microchamber can accommodate approximately 1.5 nL of a sample. The diameter of this CD-shaped device is 95 mm. As shown in Fig. 1, flow of cell suspension through the microchambers and cells is distributed into individual microchambers stochastically. Excess solution is finally drained from the microchannel by centrifugal force.

The microchannel depth of 46 μm was fabricated by deep reactive ion etching (deep-RIE) on a silicon wafer ($\phi=$ 4 inches) as follows. First, the silicon wafer was coated with positive photoresist OFPR-800 (Ohka Kogyo, Tokyo, Japan) and baked at 90 °C for 30 s. The OFPR-800 layer was exposed to UV light (HB-25103BY, Ushio, Tokyo, Japan) through a transparency mask with a previously reported pattern [17], and developed by NMD-3 (Ohka Kogyo) followed by post baking at 120 °C for 3 min. The wafer with a patterned OFPR-800 layer was etched by a deep-RIE machine (RIE-200-iPB; Samco, Tokyo, Japan) to a depth of 46 μm. The conditions used for the deep-RIE process are given in the Electronic Supplementary Material

Fig. 1 Micro fluidic design of CD-shaped device and schematic diagram of single cell isolation: (1), a large number of microchambers align along a channel; (2), the cells flow through the microchambers; and, (3), are distributed into individual microchambers. The device has 24 channels, and each channel has 313 microchambers. The width of a microchannel is 100 μm. The size of each microchamber is 300 μm (width)×200 μm (height)×46 μm (depth). Details of the CD-shaped device are given in Electronic Supplementary Material Figs. S1 and S2
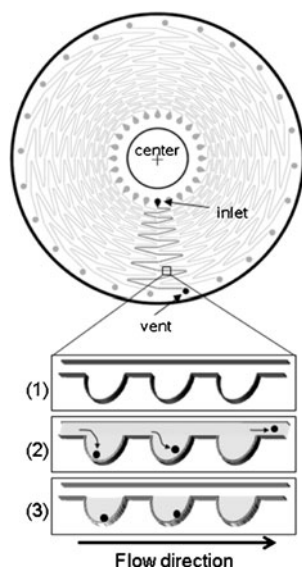
(Fig. S3). The etched wafer was washed with acetone to remove the residual OFPR-800 layer. Piranha cleaning was then performed to wash the surface of the device. Holes 2 mm in diameter for inlet and vent were bored through a glass plate by sand blasting. The etched silicon wafer was anodically bonded to the glass plate containing the holes. Anodic bonding was performed at 600 V and 400 °C for 20 min by use of an anodic bonding machine (Horivac 300 S; Horivac, Hyogo, Japan). A silicon and glass hybrid disk was used as the CD-shaped device in this study.

Reagents and sample preparation

S. enterica and E. coli were provided by the Riken BRC through the National Bio-resource Project of the MEXT, Japan. Nutrient broth with 0.5% NaCl medium for S. enterica and LB medium for E. coli were used as the culture media. Bacterial cells were cultivated in each medium overnight at 37 °C. The concentration of the cells was confirmed under an optical microscope and diluted to the desired concentration with 10 mmol L^{-1} phosphate buffer solution (PBS).

DNA amplification by PCR was performed by use of a CycleavePCR Salmonella detection kit Ver. 2.0 purchased from TaKaRa (Tokyo, Japan). This kit contains 1× Cycleave reaction mixture and 1×SIN primer/probe mix. The Cycleave reaction mixture contains polymerase, buffer and dNTP mixture. The SIN primer/probe mix is a mixture of primer and probe to detect the invA gene by PCR. In particular, the invA gene is a good candidate for Salmonella detection because it is present in all pathogenic serovars [25]. The product of this gene is essential for the organism's ability to invade mammalian cells and subsequently cause disease [26]. The probe for the PCR products was labeled with 6-carboxyfluorescein (FAM) and quencher. A 378-bp

segment of the invA gene of S. enterica was amplified by use of the kit. The increase in fluorescence from FAM occurred after the increase of the PCR product. The probe was hybridized to the PCR product then cut by RnaseH in the CycleavePCR Salmonella detection kit. Fluorescence intensity was increased by uncoupling quencher from the probe.

The reaction mixture consisted of 1×Cycleave reaction mixture, 1×SIN primer/probe mix, 0.5 U μL^{-1} Taq polymerase (TaKaRa EX Taq hot start version), and the desired concentration of the suspension of S. enterica or E. coli (100 to 400 cells μL^{-1}). The reaction mixture served for isolation of single cells and for PCR.

Single cell isolation on CD-shaped device

Before PCR of the invA gene, single cells were isolated on the CD-shaped device. One microlitre of Cycleave reaction mixture with bacterial cells was introduced into an inlet of a microchannel of the CD-shaped device. The device was then rotated counterclockwise at 1000 rpm for 10 s and 5000 rpm for 30 s by a spin-coater (model K-359 S-1; Kyowariken, Tokyo, Japan). The reaction mixture containing cells was separated into each microchamber and single cells were isolated into some of the microchambers on the CD-shaped device. After isolation, the microchambers, each with a single cell, were completely isolated from each other by air [17].

DNA amplification and detection

In this study, 100 to 400 cells μL^{-1} of S. enterica were examined in individual microchannels on the same device. To prevent evaporation of the reaction mixture after single cell isolation, the inlet and vent were sealed tightly with a butyl gum polymer (Blu-Tack, Bostik Findley, Australia), which quite effectively maintained the microchannel moist during PCR cycles. The device was placed on to the stage of a thermal cycling system, which was custom-made by Astec (Tokyo, Japan). This stage was shaped to fit to the CD-shaped device. The inlet and vent were covered tightly with the thermal cycling system's lid to prevent evaporation of the reaction mixture. On the micro fluidic devices for PCR, the generation of micro bubbles in the channel by heat is a common problem. Micro bubbles in micro fluidic devices are generated by evaporation of solution in the microchannel during PCR. On our device, no solution remained in the microchannel after cell isolation (Fig. 1.3). The solutions in the microchambers were completely separated by air on our device. The inlets and vents of the microchannel were then tightly closed with the lid of the thermal cycler to prevent evaporation of the solution. Micro bubbles did not occur in our device because the solution

could not evaporate because of an increase of micro-chamber pressure by heating during PCR cycles. Thermal cycling was initiated at 95 °C for 2 min to lyze the cells, followed by 20 to 50 cycles of 95 °C for 5 s, 55 °C for 10 s, and 72 °C for 10 s. Immediately after the cycles, the fluorescence intensity of PCR products on the device was determined through an optical filter and analyzed by use of a commercially available image analyzer (LAS-3000; Fujifilm, Tokyo, Japan). The fluorescence intensity caused by PCR was obtained by subtraction of the background fluorescence intensity which emanated from the liquid-free surface.

Conventional PCR was performed in a PCR tube. The reaction mixture consisted of PCR reagents and bacterial cells. Thermal cycling was then performed by a PCR cycler (Gene Amp PCR System 9600; TaKaRa) with the same thermal cycling as in our device. The fluorescence intensity of PCR products in the tube was acquired and analyzed with the same image analyzer as for the microchambers.

Results and discussion

Efficiency of S. enterica isolation on CD-shaped device

To confirm whether the solution was separated to micro-chambers in all microchannels in the same manner, the microchambers on the CD-shaped device were examined by use of a microscope after separation of a dye solution at 5000 rpm for 30 s, Most microchambers on all micro-channels contained an equal amount of dye solution. The average ratio of microchambers containing the dye solution of all the microchambers was 98.42±0.77%. This result indicates the feasibility of separating samples in micro-chambers in the CD-shaped device at the same time for parallel experiments. In this study, spin-speed was an important conditions for separation of the solution on the device. At a slower spin-speed, the centrifugal force acting on the liquid in each microchamber weakens. Therefore, almost all liquid flowed downstream without remaining in the microchamber because of the viscosity of the solution. At a faster spin-speed, the solution slipped out of the microchambers without displacing the air in the micro-chambers. As a result, little solution remained in the microchambers, with air bubbles at their bottom. In this CD-shaped device, 5000 rpm was the best condition for keeping liquid in the microchambers.

Separation of an S. enterica cell suspension was demonstrated in the CD-shaped device, as we confirmed that the solution could be separated into microchambers at a rate of approximately 98% for all microchannels. After separation of the S. enterica cell suspension at 5000 rpm for

30 s, the probability of exiting of S. enterica cells in a microchamber was supposedly the same as the probability of exiting of S. enterica per microchamber volume because the specific gravity of S. enterica cells was not very different from that of 10 mmolL^{-1} PBS. The distribution of S. enterica on the CD-shaped device was expected to be a Poisson distribution. The probability of a variable number, k, of trapped particle(s) in a chamber is described in Eq. 1:

$$P(N = k) = \frac{e^{-m}m^k}{k!} \tag{1}$$

where m indicates the average number of cells in a microchamber. In this study, each microchamber could contain 1.5 nL S. enterica suspension. When the density of the cell suspension is 400, 200, and 100 cells μL^{-1}, m is 0.6, 0.3, and 0.15, respectively.

In this study, the efficiency of isolation of S. enterica cells was examined. The separated cells on the device were observed under an optical microscope (Electronic Supplementary Material Fig. S4). The distribution of S. enterica cells was almost constant along the channel. No bias in the distribution of S. enterica cells was confirmed from upstream to downstream.

The efficiency of S. enterica cell isolation is shown in Table 1. One hundred microchambers were observed under

Table 1 Relationship between the efficiency of cell isolation and the Poisson distribution. The efficiency of S. enterica isolation on the CD-shaped device was compared with the theoretical value from the Poisson distribution

Density of cell suspension (cells μL^{-1})	Number of cells (cells per microchamber)	Theoretical value (%)	Experimental value (%)
400	0	54.9	55.3±2.33
	1	32.9	31.7±1.86
	2	9.9	10.7±0.88
	3	2.0	2.3±0.67
	4	0.3	0.3±0.33
200	0	74.1	73.7±2.40
	1	22.2	21.3±1.86
	2	3.3	4.3±0.33
	3	0.3	0.7±0.33
	4	0.0	0.0±0.00
100	0	86.1	85.3±2.60
	1	12.9	13.3±2.03
	2	1.0	1.3±0.67
	3	0.0	0.0±0.00
	4	0.0	0.0±0.00

"Theoretical value" indicates the value calculated from the Poisson distribution. "Experimental value" indicates the experimental value ± the standard error. The density of S. enterica was 400, 200, or 100 cells μL^{-1}

an optical microscope in each experiment. The observed number of *S. enterica* cells in each microchamber agreed with the value evaluated from the Poisson distribution, irrespective of the density of the cell suspension. The isolation efficiency of *S. enterica* was consistent with the results we had previously reported using beads [17]. For the lower density, the efficiency of single cell isolation was higher. We decided that the microchamber sizes should be 300 μm×200 μm in this study, because an imaging analyzer with higher resolution was necessary for smaller microchambers. However, if the microchamber was smaller, the efficiency of single cell isolation would be much higher in the device. Isolation of single cells and prevention of evaporation of the solution in microchambers were possible in the device with a smaller microchamber whose dimensions were 180 μm×120 μm. The fluorescence intensity of PCR products in several smaller microchambers was same as that observed in this study. Basically, the amount of PCR product originating from a single *Salmonella* cell should be the same, despite of the volume or size of the microchamber. However, insufficient resolution of the imaging analyzer ruled out the detection of PCR product in a smaller microchamber. In the case of a sample containing more than one bacterium, some bacteria could hardly be distinguished under a microscope. To distinguish these cells, amplification of a specific gene would be very useful.

Optimization of the number of PCR cycles on CD-shaped device

Before detection of *S. enterica* cells on the CD-shaped device, the DNA sequence of the *Salmonella invA* gene was amplified by using the Cycleave PCR Salmonella detection kit. The microchannels of the device were filled with the reaction mixture with *invA* as the positive control or sterilized water as the negative control. In this experiment, we used the positive control (*invA*, 1×10^4 copies μL^{-1}) provided in the Cycleave PCR Salmonella detection kit. The final concentration of *invA* in the device was 400 copies μL^{-1}. In this experiment, the FAM probe in the PCR reagent was used to detect amplification of the specific gene without electrophoresis. In order to detect the gene dosage in microchambers, it is necessary to optimize the number of PCR cycles on the device. Amplification of the *invA* gene was performed by changing the number of PCR cycles. The fluorescence intensity of 20 parts of the microchannel on the device was detected randomly after each cycle. Figure 2 indicates the difference in fluorescence intensity between the positive and negative controls for each number of PCR cycles. Although the fluorescence intensity after 20 cycles was small, it increased linearly from 30 to 50 cycles. In this experiment the increasing
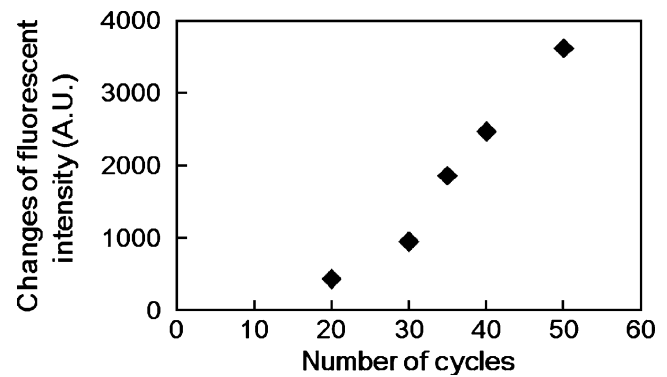

Fig. 2 Different fluorescence intensities of the *invA* positive control and the *invA* negative control for different numbers of PCR cycles

tendency of the fluorescence intensity on the device was similar to that of conventional PCR by the use of a PCR tube. The number of PCR cycles should be enough to detect the product on the basis of the fluorescence intensity. The difference in fluorescence intensity in gene dosage could not be confirmed by saturation of fluorescence when too many cycles were used. In common quantitative PCR, fluorescence intensity was saturated after 50 PCR cycles in most situations. Therefore, we decided to detect the *invA* gene after 40 cycles in this study, because it was expected that fluorescence intensity should depend on the number of cells in a microchamber.

Single-cell PCR on CD-shaped device

To realize PCR of a single cell on the CD-shaped device, we performed direct amplification of DNA from *S. enterica* cells although conventional PCR was performed by using DNA extracted from cells. In the CD-shaped device, the cell should be lyzed in the microchamber after its isolation. Before PCR of cells on the device, we confirmed that DNA could be amplified from cells directly in a PCR tube. *S. enterica* cells were lyzed in the reaction mixture immediately followed by conventional PCR cycles in a tube. The increase in fluorescence after DNA amplification was clearly confirmed (Electronic Supplementary Material Fig. S5).

Next, we performed PCR of cells on the CD-shaped device. Reaction mixtures containing a suspension of 200 or 400 *S. enterica* cells μL^{-1} were examined. Each reaction mixture was separated into microchambers along the microchannel in the device by spinning. After separation of the *S. enterica* suspension, isolated cells were lyzed at 95 °C for 2 min, followed by continuous PCR on the device. The final amount of PCR product depended on the initial amount of template sequence (*invA* gene) in a microchamber, and the fluorescence intensity was proportional to the amount of PCR product. Thus fluorescence intensity from microchambers containing several *S. enter-*

ica cells was expected to be higher than from those containing a single *S. enterica* cell.

The fluorescence from 57 microchambers in the device was measured (Fig. 3). Their fluorescence intensities were distributed from 156 to 1016 and the intensities were distributed randomly from upstream to downstream. There was no bias between upstream and downstream as the cells were distributed equally from upstream to downstream. It was expected that the fluorescence intensities would form groups based on the number of cells after PCR according to a Poisson distribution. The fluorescence intensity of the negative control was lower than 350. Therefore, a microchamber with approximately 350 or lower fluorescence intensity was identified as that containing no *S. enterica* cell(s). To understand what the fluorescence intensities indicated, the fluorescence intensity data were organized into a histogram (Fig. 4). Figure 4 (a) indicates the number of 400 *S. enterica* cells μL^{-1}. In this histogram, four groups of fluorescence intensities can be recognized. The fluorescence intensities of the first, second, third, and fourth groups were approximately 250, 450, 650, and 1000, respectively. The fluorescence intensity after PCR depends on the amount of *invA*, which should be proportional to the number of cells. These groups of fluorescence intensities were believed to contain 0, 1, 2, or 3 cells in each microchamber. In this experiment, the first and second group overlapped. The group with fluorescence intensity lower than 350 indicated no cells because the maximum fluorescence intensity of the negative control was 350. The ratios of the number of cells (0, 1, 2, and 3 cells) calculated from the histogram in Fig. 4(a) were 42.1, 45.6, 10.5, and 1.8%, respectively. Those ratios were fitted to the value of cell distribution in the device. At a high density of *S. enterica* cells, the ratio of multiple cells in a microchamber was slightly high in the device. Therefore, to confirm the fluorescence intensity of the microchamber containing a single cell after a definite increase in PCR, we performed the experiment by
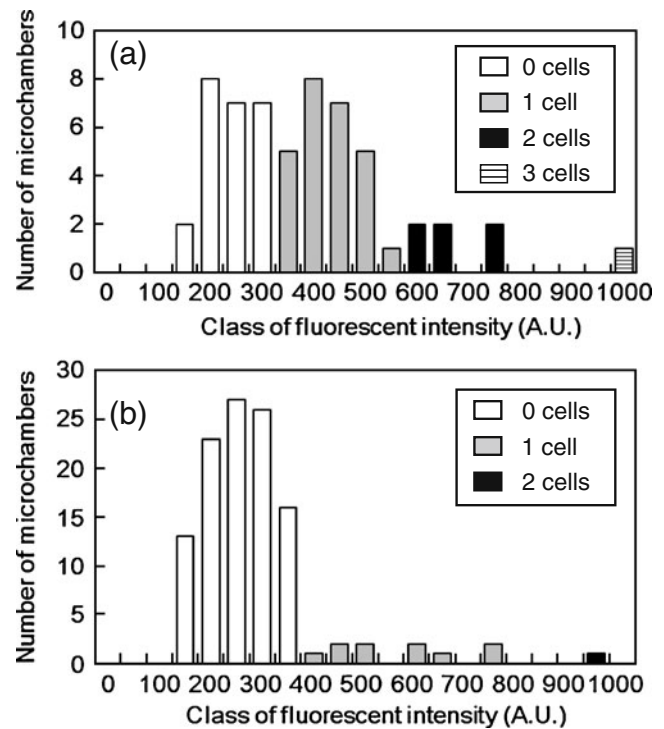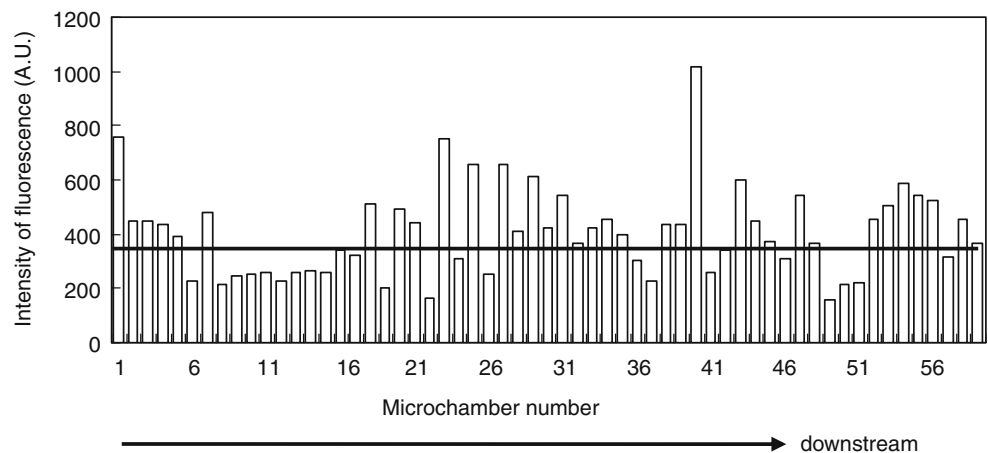

Fig. 4 Histograms of fluorescence intensities for different densities of *S. enterica*. Histograms indicate the number of microchambers in different groups of fluorescence intensity after 40 PCR cycles on the CD-shaped device. The density of *S. enterica* was: (a) 400 cells μL^{-1}, (b) 200 cells μL^{-1}. The *white*, *gray*, *black*, and *striped bars* indicate 0, 1, 2, and 3 cells, respectively

using a sample with 200 *S. enterica* cells μL^{-1} (Fig. 4b). In this histogram, three groups of fluorescence intensities were recognized. The fluorescence intensities of the first, second, and third groups were approximately 250, 550, and 950, respectively, indicative of 0, 1, or 2 cells in each microchamber. The ratios of the number of cells (0, 1, and 2 cells) calculated from the histogram in Fig. 4(a) were 90.5, 8.6, and 0.9%, respectively, somewhat lower than the value of cell distribution in the device. Although the first and second groups in Fig. 4(a) overlapped, such

Fig. 3 Fluorescence intensities after PCR on the CD-shaped device. For 400 *S. enterica* cells μL^{-1}, the fluorescence intensity of 57 microchambers is shown to range from 156 to 1016. The *horizontal line* indicates the maximum fluorescence intensity of the negative control

overlap was not recognized in Fig. 4(b). At a density of *S. enterica* cells, DNA amplification from a single cell by PCR was definitely confirmed on the device. We performed the same experiments several times to confirm repeatability. The histograms of the fluorescence intensities showed almost the same values each time for the device. In this experiment, we confirmed approximately 100 microchambers of one microchannel in the device because of the limitation of the imaging area which was acquired at once by an image analyzer. We were able to measure the fluorescence intensities of 7512 microchambers on the device by developing an imaging system with a larger imaging area and higher resolution. In the future, our device will be coupled with a high-throughput detection system for many single cells.

Selectivity of specific cells on the CD-shaped device

To examine selectivity between *S. enterica* and *E. coli* by PCR on our device, DNA amplification of the *invA* gene was performed against *E. coli* which does not harbor this gene. For 200 *E. coli* cells μL^{-1}, the fluorescence intensities were lower than 250 after PCR (Fig. 5a). These low fluorescence intensities implied there was no amplification of the gene because the maximum fluorescence intensity of the negative control was approximately 350. No increase in fluorescence was observed as a result of the presence of *E. coli*. Increased fluorescence intensity was indicative of the presence of the *S. enterica invA* gene.

Next, a mixture containing 100 *S. enterica* cells μL^{-1} and 100 *E. coli* cells μL^{-1} was examined. As shown in Fig. 5(b), two groups of fluorescence intensities were recognized. The first and second groups were approximately 250 and 600, respectively. In this experiment, the fluorescence intensity of three microchambers increased in contrast with the result for the 200 *E. coli* cells μL^{-1}. The first group of fluorescence intensities was assumed to be the group with no cells or only *E. coli* cells in each microchamber. The second group of fluorescence intensities was considered to be from microchambers containing a single *S. enterica* cell, because the fluorescence intensities were comparable with that from the single *S. enterica* cell group in Fig. 4(b), in which 200 *S. enterica* cells μL^{-1} was used. These results indicate that *S. enterica* could be identified from a mixture of *S. enterica* and *E. coli* by PCR on the CD-shaped device. Herewith, the detection of a specific gene by PCR on the device was demonstrated and the detection of other specific genes is most likely possible with the same method by using different primer pairs.

This device could be used to determine how many bacterial cells exist in a sample by PCR. Furthermore, the CD-shaped device can be easily handled because it is a very simple format without using a micro pumping system. In
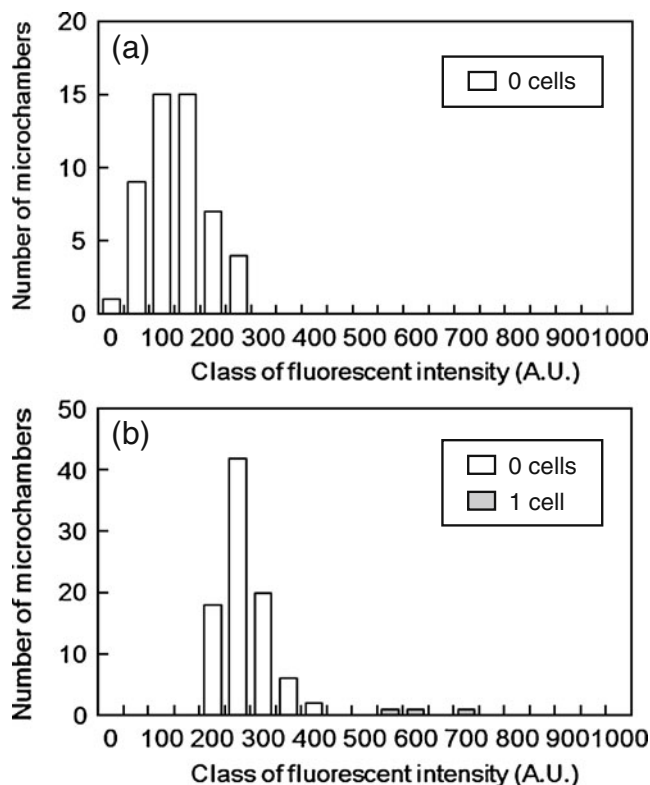

Fig. 5 Selectivity for *invA* gene on CD-shaped device between *S. enterica* and *E. coli*. These histograms indicate the number of microchambers in different groups of fluorescence intensity after 40 PCR cycles on the CD-shaped device. The density of bacterial cells was: (a) 200 cells of *E. coli* μL^{-1} and (b) 100 cells of *S. enterica* μL^{-1} +100 cells of *E. coli* μL^{-1}. The *white* and *gray bars* indicate 0 and 1 cells, respectively

the various reported systems of single cell isolation and/or PCR on a micro device, a micro pumping system was necessary to distribute solution [27–29], and control of the micro pumping system is still not easy achieve. On the other hand, as for the single cell isolation on our CD-shaped device, the isolation speed is only 5000 rpm for 30 s, which can be enabled by the driving mechanics of a CD drive of a standard personal computer. Therefore, this CD-shaped device for single cell isolation and PCR is expected to be promising to various fields in the future.

Conclusion

After single cell isolation, DNA amplification of the *Salmonella*-specific *invA* gene from single *S. enterica* cells was demonstrated on the CD-shaped device. Evaporation of the small amount of reagent was avoided by fabricating of a device from silicon wafer and glass. When the *S. enterica* cells were isolated on the CD-shaped device, a Poisson distribution was observed. For 200 *S. enterica* cells μL^{-1} or less, DNA amplification of the *invA* gene from a single cell

was successful on the device. The selective detection of *S. enterica* was achieved by PCR from a mixture of *S. enterica* and *E. coli*.

This device is a very simple format which can be used without a micro pumping system. Our CD-shaped device is very useful, because the rotation speed is only 5000 rpm for 30 s, which would be enabled by the driving mechanics of a CD drive of a standard personal computer. Therefore, this CD-shaped device is expected to be utilized in a wide range of fields in the future.

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