OPTICAL TEMPERATURE CONTROL OF MULTIPLE MICROHEATERS USING DIGITAL MICROMIRROR DEVICE

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ABSTRACT

We developed a novel technique for local temperature control of the multiple microheaters on a microchip with a 2D projected pattern of the IR laser using the DMD (Digital Micromirror Device: Texas Instruments). The DMD contains a rectangular array of about 0.5 million hinge-mounted microscopic mirrors or more. Each angle of micromirrors is controlled based on the grayscale picture on the PC monitor. We used it to pattern the IR laser. The reduced projection of the patterned laser beam is irradiated to the evenly divided ITO(Indium Tin Oxide) thin films on the glass plate through the optical lens. Here we applied this method to the immobilization of multiple microorganisms by the thermosensitive hydeogel to assay their individual properties. The hydrogel solution around the irradiated microheater was gelled. In experiment, we confirmed gelation of arbitrarily-selected microheaters, and we succeeded in immobilization of target cells in-situ on a chip.

1. INTRODUCTION

Most microorganisms visible under a microscope are viable but do not grow to form colonies visible on plates. Of such microorganisms, some are cells of known species for which the cultivation conditions used happen not to be suitable or are cells that have entered an unculturable state and the others are of unknown species that have never been cultured before suitable methods [1]. There are great demands to investigate unknown properties of microorganisms. Analysis based on the batch culture is commonly used.

High-throughput separation of viable cells with desired characteristics from a large heterogeneous population is needed before conditions for the strains or for unidentified microorganisms can be found. Single-cell separation with a mechanical micromanipulator is not easy if the sample cells are suspended in a liquid. Flow cytometry is widely used for this purpose [2-6]. However, most conventional separation processes are sequential, so it is impossible to compare multiple objects simultaneously before separation, and positional information is lost by separation.

Unlike in the previous approach, we removed obstacles around the target rather than transporting the target. We employed a novel method to isolate the target by thermal gelation [7-9]. The targets are fixed in the gel and obstacles are washed out by a cleaning flow. By on-chip separation and monitoring, the place of isolation is used for several purposes. This concept is very different from the conventional approach. To control the local temperature in the microchannel, we used an ITO (Indium Tin Oxide) electrode as a heater. However, it is difficult to control multiple heaters independently on a chip.

In this paper, we developed a novel technique for local temperature control of the multiple microheaters on a microchip with a 2D projected pattern of the IR laser using the DMD (Digital Micromirror Device: Texas Instruments). The DMD contains a rectangular array of about 0.5 million hinge-mounted microscopic mirrors or more. Each angle of micromirrors is controlled based on the grayscale picture on the PC monitor. We used it to pattern the IR laser. The reduced projection of the patterned laser beam is irradiated to the evenly divided ITO thin films on the glass plate through the optical lens. The ITO film is used to absorb heat and divided into small blocks for thermal insulation and positioning of cells by laser tweezers. We succeeded in local temperature control based on the grayscale picture on the PC monitor. We call the small ITO tile as the microheater. The proposed method can be used for several biochemical experiments, such as thermochemical reaction and culture, on a chip. Here we applied this method to the immobilization of multiple microorganisms by the thermosensitive hydeogel to assay their individual properties. The hydrogel solution around the irradiated microheater was gelled. In experiment, we confirmed gelation of arbitrarily-selected microheaters, and we succeeded in immobilization of target cells in-situ on a chip.

2. MATERIAL AND METHOD

Poly(*N*-isopropylacrylamide) (PNIPAAm) is a thermo-sensitive hydrogel. PNIPAAm 10w% solution gels around 32°C [10]. PNIPAAm has a property of sol-gel reversible transformation (Fig. 1). The maximum velocity, that the hydrogel can resist in the microchannel flow, is under 200 μm/s [9]. PNIPAAm is harmless to the microbe or human body, so this is suitable for manipulation of viable cells or microorganisms.

Figure 2 shows the schematic of immobilization method of multiple microbes with PNIPAAm solution and ITO tiles. PNIPAAm solution mixed with specimens is injected in the microchip. There are multiple ITO tiles on the glass plate at the bottom of the chip. Laser beam patterned by the DMD is irradiated to the ITO tile for heating. This laser is focused by an objective lens to increase the energy density of the laser at

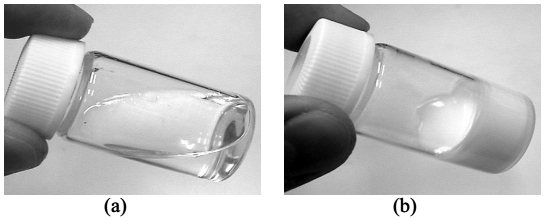

Fig. 1 A photograph of PNIPAAm 10w% solution in macro scale. (a) before gelation (b) After gelation (30°C)

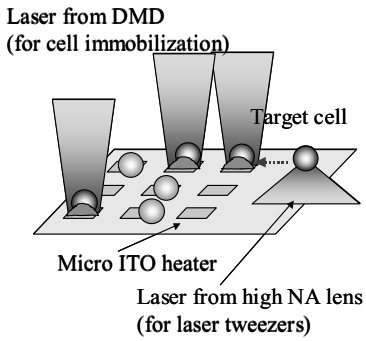

Fig. 2 Outline of the proposed immobilization method. The target cells are trapped by the hydrogel.

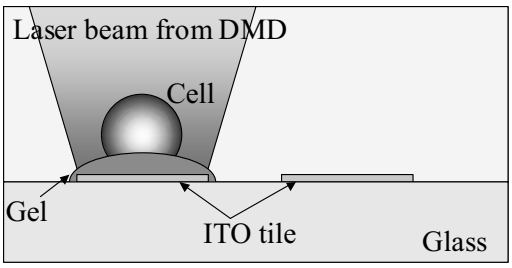

Fig. 3 Schematic of cross section of ITO tile (Side view). The target cell is trapped by the gel.

the surface of the ITO tile. The solution around the tile was gelled by heating with the laser, and the cells around the tile are immobilized by the gel. We employ laser tweezers for positioning the target samples as shown in Figure 2. Figure 3 shows a schematic of cross section of ITO tile. The ITO thin film is deposited on the slide glass for absorption of the IR laser. With this method, we succeeded in immobilization of multiple target microbes.

3. SYSTEM

We used a projector (Plus-vision) to pattern the laser beam by the DMD. The laser beam from the laser source is reflected by the two mirrors, and it is divided by the DMD. Arbitrary pattern is generated by the DMD based on a signal from the PC. Therefore, we can get an image of IR laser which is homothetic to the image of the PC monitor. The DMD has been used for manipulation of microobject [11] or photopolymerization [12]. However, application to optical temperature control and cell immobilization has not been proposed before.

Figure 4 shows a schematic of the optical system. Both manipulation and immobilization works are operated on the inverted microscope. Two IR lasers (Nd:YVO_4, 1064 nm) are installed in the inverted microscope. One is used for laser tweezers with a high-power objective lens (x100, NA: 1.35). The other is used for optical heating with a low-power lens (Ex: x20, NA: 0.45). Here we propose to employ divided ITO tiles as microheaters. The patterned laser beam was irradiated to the multiple microheaters. Figure 5 shows the micrographs of the ITO tiles. We make two types ITO chips. A long gap (8 μm) type is used with laser tweezers for fine position control of the target. Another one, short

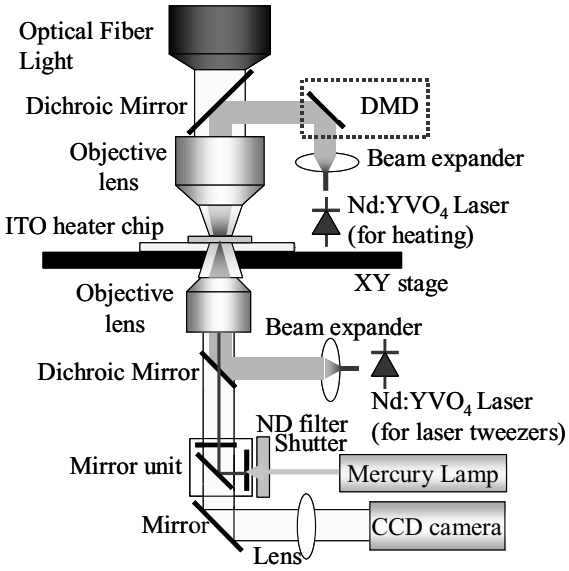

Fig. 4 Schematic of the optical system.

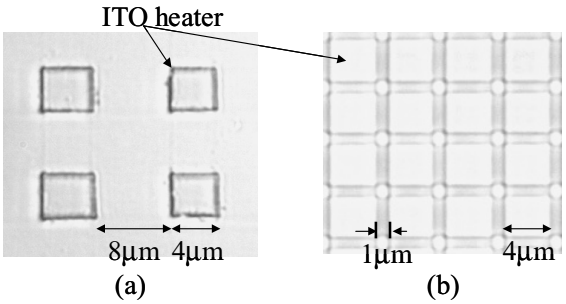

Fig. 5 ITO tiles (Top view). (a) 4 um x 4 um tiles with 8 um pich. (b) 4 um x 4 um tiles with 1 um pitch

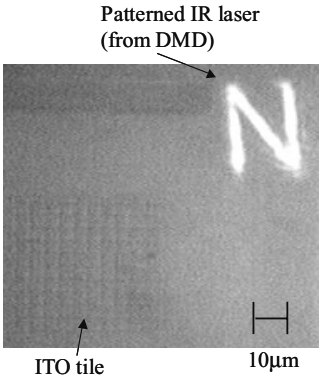

Fig. 6 Micrograph of patterned Nd:YVO4 laser. The laser describes a character "N".

gap(1μm), is used for selective immobilization of the target microbes without laser manipulation. ITO thin film is an alloy with high electric conductivity and its sheet resistance is around 30 Ω/sq. Moreover, it is transparent to visible light (80%) and near-infrared ray (70%). Hence, ITO is favorable for electrode use under optical inverted microscope. A tile-shaped processing method is effective for the heat insulation, because the thermal conductivity of the glass (about 1.38 [W m^{-1} K^{-1}]) is much smaller than that of the ITO (8.18 [W m^{-1}

K $^{-1}$] [13][14]). Figure 6 is a micrograph of the patterned laser monitored with the CCD camera. We can see the laser beam describes a character "N".

4.MEASUREMENT

We measured the relation between width of laser irradiation area and width of gelation area. Figure 7 shows schematic of microchip and definition of laser irradiation area and gelation area. The projected laser beam is irradiated to the ITO heater and flat ITO surface. Figure 8 shows the result of relationship between laser irradiation area and gelation area at the stationaly phase. The lowest gelation temparature of the PNIPAAm 10w% solution is 32°C, and the room temparature during the experiment was 26.1°C. The solution started to gel when the width of laser irradiation is set about 2.4 μm and the laser power was 0.105 mW. The gelation area of flat ITO surface is larger than that of ITO tile. This result shows the ITO tile is effective for the heat insulation. Analysis of temperature distribution is our next work.

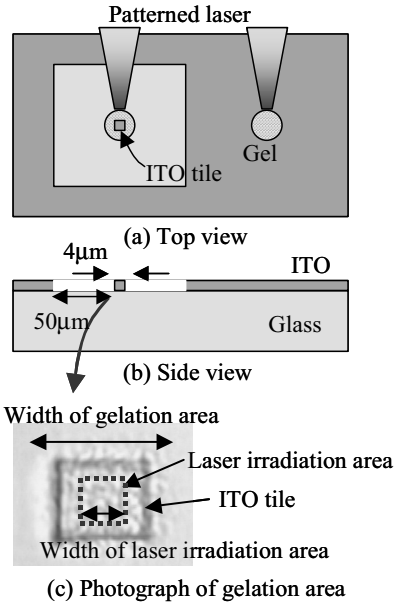

Fig. 7 Definition of laser irradiation area and gelation area.

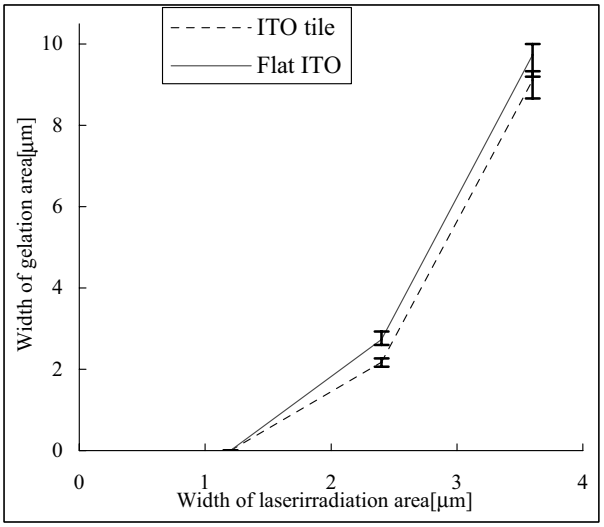

Fig. 8 Relationship between laser irradiation area and gelation area. The projected laser beam is irradiated to the center of ITO heater and flat ITO surface.

5. EXPERIMENT

Figure 9 shows the result of the individual gelation of the ITO tiles. The ITO tile chip is filled by the PNIPAAm 10w% solution. The tile is 4 μm square and the gap is 8 μm. We can gel arbitrary ITO tile using laser beam patterned with the DMD as shown in Fig. 6.

Figure 10 shows the cell immobilization using ITO tile and laser tweezers. The solution mixed with PNIPAAm 10w% solution and yeast cells (4μm ϕ) is injected in the ITO tile chip. Laser beam from high NA lens is irradiated to the selected yeast cell for laser manipulation. The ITO tile absorbs the laser and the laser is focused in high power laser, so each tiles is isolated and the trajectory of the target cell has to be determined carefully to avoid the ITO tile (Fig.10 (a)) [15][16]. The target cell is positioned by the laser tweezers and released near the ITO tile (Fig.10 (b)). Then the laser beam patterned with the DMD was irradiated around the ITO tile to gel, and the target yeast cell was immobilized by the gel as shown in Figure 10 (c).

Figure 11 shows the immobilization of multiple selected cells. The tile is 2 μm square and the gap is 4 μm. The solution mixed with yeast cell and PNIPAAm 10w% solution is injected. The ITO tile at center and center of bottom line are gelled and two yeast cells are immobilized with the gel.

In this report, the diameter of gelation area is about 30 μm

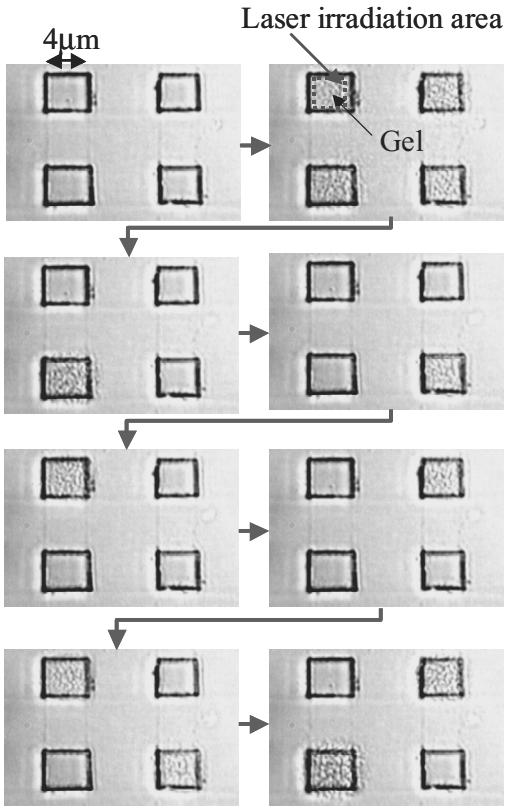

Fig. 9 Experiment of local heating by laser with DMD.

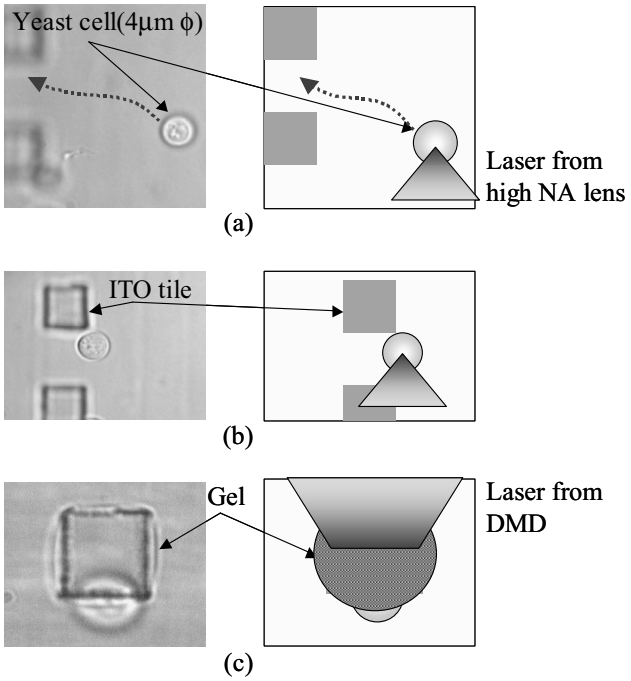

Fig. 10 Immobilization of an individual cell. The position of the target can be adjusted by the laser tweezers.

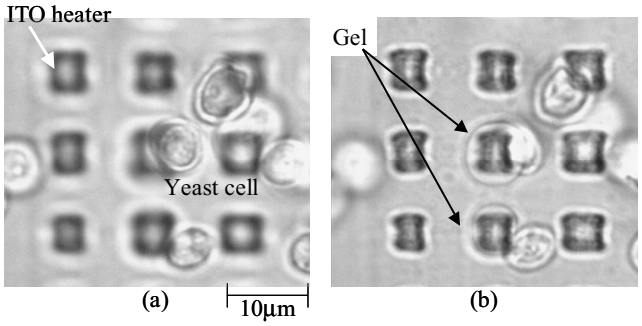

Fig. 11 Immobilization of multiple selected cells.

ϕ because the IR laser has a Gaussian distribution. In the future work, we will employ a high power laser and a beam homogenizer to adjust the laser beam distribution.

6. SUMMARY

We have developed a desktop system for in-situ immobilization of microorganisms. The specimen is fixed by the thermal gelation of a poly(*N*-isopropylacrylamide) solution. We succeeded in local temperature control based on the grayscale picture on the PC monitor. We call the small ITO tile as the microheater. The proposed method can be used for several biochemical experiments, such as thermochemical reaction and culture, on a chip. Here we applied this method to the immobilization of multiple microorganisms by the thermosensitive hydeogel to assay their individual properties. The hydrogel solution around the irradiated microheater was gelled. In experiment, we confirmed gelation of arbitrarily-selected microheaters, and we succeeded in immobilization of target cells in-situ on a chip.

We succeeded in the immobilization of yeast cells on a chip. This concept is different from the conventional approach. The method can be easily extended to the parallel culture of cells on a chip. Several different incubation conditions can be investigated on a single chip on the basis of single-cell reactions.

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