AN OPTO-THERMOCAPILLARY CELL MANIPULATOR

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ABSTRACT

Single yeast cells in an agarose solution were patterned using the optically controlled thermocapillary flow surrounding a vapor bubble in the solution. After cell patterning, the agarose solution was gelled, and the cells were able to grow in this 3D biocompatible scaffold. Of the 12 cells in the initial pattern, 11 underwent multiple cell divisions during culturing for nine hours. This opto-thermocapillary manipulation provides a unique, cell-friendly method of patterning for cell and tissue cultures and associated research.

KEYWORDS

Thermocapillary flow, cell manipulation, cell patterning

INTRODUCTION

Single-cell manipulation is traditionally done by micromanipulators [1], macro-scale instruments equipped with micro-scale end-effectors. However, these systems require skilled operators and have limited throughput. The programmable manipulation of multiple cells can be achieved by optical tweezers systems [2]. Cell manipulation using optical tweezers is programmable and can be automated, but illuminating cells with intense laser light can cause cell damage [3]. Another programmable optical cell manipulation tool is optoelectronic tweezers (OET), which uses light to modulate electric field gradients, creating dielectrophoretic force that can move cells [4]. The light used for OET is much less intense than that of optical tweezers; however, the electrical properties of the cell media can affect manipulation [5].

In this report, optically induced thermocapillary flow is utilized for single-cell manipulation. This technique is compatible with a standard optical tweezers setup, but its manipulation force is independent of the objects' optical properties, and does not require direct illumination of the cells under manipulation. No electric field is applied, making this system compatible with a wide range of physiological media. In addition, the system uses an open fluidic chamber for the cell manipulation, which facilitates cell culture and harvesting after the cell patterning. Similar techniques were previously reported for the manipulation of glass beads [6], [7] and the patterning of polystyrene beads [8]. The technique described here uses a 980-nm near-infrared laser to generate smaller bubbles (less than 5 μm in diameter) than those in refs. [6] and [7], facilitating smaller-scale thermocapillary flow and higher resolution, and enabling the manipulation of single microparticles or cells.

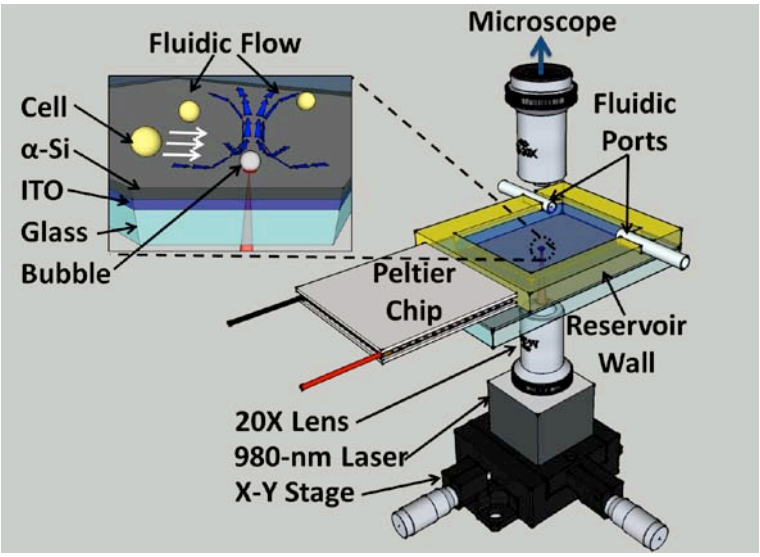

Fig. 1: Opto-thermocapillary cell manipulation system. Cells reside within an open fluidic reservoir with polyimide-tape walls 1.2 mm high. A Peltier chip is used for controlling the reservoir temperature. The inset shows the layers of the substrate at the bottom of the open fluidic chamber, and the toroidal thermocapillary flow surrounding a bubble in the liquid.

In the current system (Fig. 1), the reservoir is built on a 1.1-mm-thick glass substrate that is coated with a 200-nm-thick indium tin oxide (ITO) layer and a 1-μm-thick amorphous silicon (a-Si) film. A 980-nm diode laser is focused on this absorbing film, with an intensity of 508 kW/cm^2 at the focal point. The substrate converts 70% of the laser intensity into heat [7]; this localized heating generates the bubbles used for manipulation. The temperature

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difference at the bubble surface leads to a toroidal thermocapillary flow that moves towards the bubble near the surface of the substrate, and then circulates upwards and away from the bubble after being accelerated by the thermocapillary effect at the bubble surface. Cells or other microparticles on the substrate are pulled towards the center of the toroidal flow when they are within its range. The target objects, which always move towards the bubble located in the center of the laser spot, can be pulled in the desired direction by scanning the laser on the substrate. For example, a 10-µm-diameter polystyrene bead can be pulled around a feature on the substrate by moving a pulsed laser relative to the substrate (Fig. 2a). The laser pulse used has a width of 60 µs at a frequency of 60 Hz. Between each laser pulse, the bubble dissolved into fluid quickly due to the dropping vapor temperature and high Laplace pressure (Fig. 2b). The following laser pulse generates a new bubble in the desired direction of movement, which continues to pull the target objects.

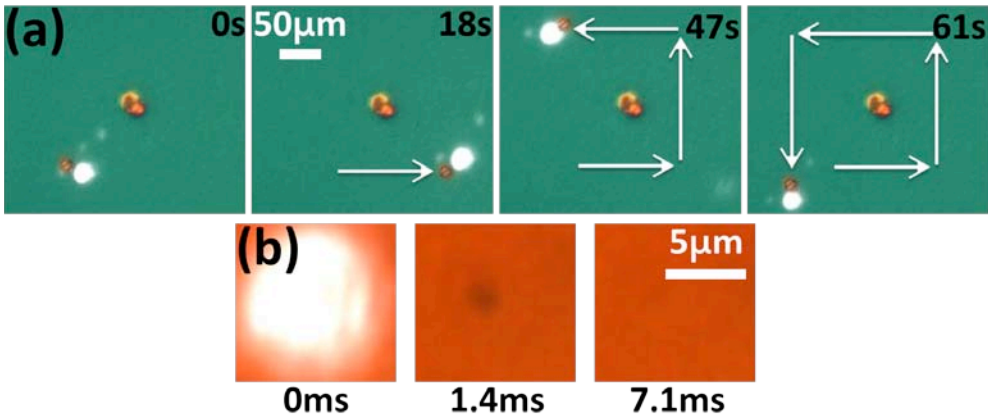

Fig. 2: Opto-thermocapillary manipulation (a) Manipulation of a 10-µm-diameter polystyrene bead in 1.5% agarose solution. The bead (the smaller object in the image sequence) was pulled around a feature on the substrate (the larger object in the center of the images). (b) A 2-µm-diameter bubble was generated after one laser pulse, and dissolved within 7.1 milliseconds.

CELL MANIPULATION AND PATTERNING

Pulling velocity increases as the laser pulse width and pulse frequency increase (Fig. 3a), helping with long-range trapping and rapid particle manipulation. However, these stronger flows reduce the manipulation resolution, as beads that are farther away from the laser focal point are attracted. This is shown in the Figure 3b, which overlays 2000 video frames captured over a period of 20 seconds, with the laser positioned among 10-µm-diameter beads. Thus, the laser pulse widths can be adjusted as desired to achieve stronger forces or precision micromanipulation.

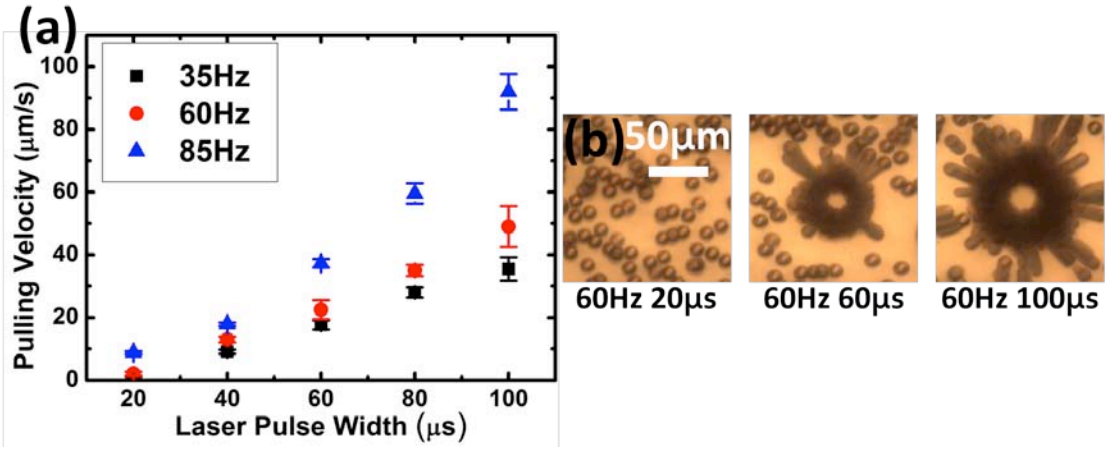

Fig. 3: (a) Measured velocity while pulling 10-µm- diameter polystyrene beads in 1.5% agarose solution. (b) Composite images over 20 seconds, taken under the same conditions as in (a). As pulse width increases, beads are pulled in towards the laser over a larger area.

Cell patterning was demonstrated by arranging yeast cells into an "H" (for Hawaii) in 1.5% ultra-low-gelling-temperature agarose solution (Fig. 4). Yeast was chosen in order to have rapid cell division. The yeast cells were collected within a 200-µm-radius circular area. Due to higher temperatures and surface tension at the bubble surface, the yeast were kept at least 25 µm from the laser spot at all times. This distance keeps the yeast at a safe temperature of less than 35 ºC, as verified by empirical measurements using a thermocouple. A laser pulse frequency of 60 Hz was used throughout the manipulation (Fig. 4a, 4b). The laser pulse width was set to 60 µs for transporting cells

to the assembly sites, and 20 μs for fine adjustments of cell positions. The final pattern contains 12 yeast cells. The average yeast diameter is 8 μm and the average distance between each pair of yeast after assembly is 43 μm. After the cell patterning was completed (Fig. 4c), the agarose was gelled (Fig. 4d) by lowering the temperature of the fluidic chamber to 15 °C for 10 minutes. This ensures that the assembled yeast cells remain in place, and the agarose hydrogel acts as a scaffold for 3D cell growth. Following agarose gelation, yeast peptone dextrose solution was added to the chamber through fluidic ports, and the chamber temperature was raised to 32 °C for yeast culturing. Of the 12 cells in the initial pattern, 11 underwent multiple cell divisions.

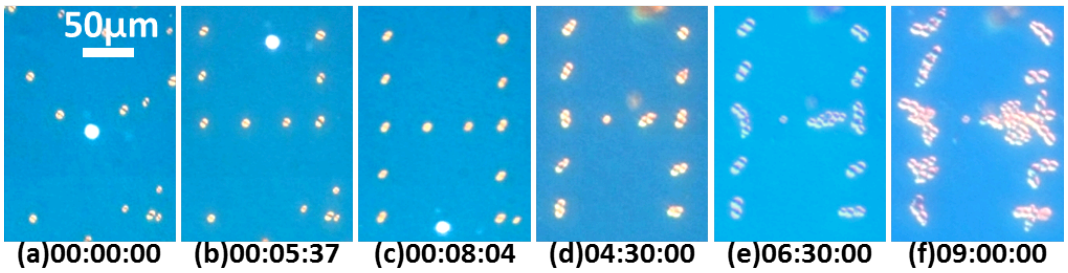

Fig. 4: (a-c) Assembly of yeast cells into an "H" in agarose solution, (d-f) Gelation of agarose and culturing for 9 hours. The solution was maintained at 15°C during gelation and at 32 °C at all other times.

The average single yeast manipulation velocities are 10 μm/s and 4 μm/s for the 60 μs and 20 μs laser pulse widths, respectively. This relatively low velocity can be improved in the future. Currently, the assembly process is controlled manually, and the current manipulation speed gives the operator enough time to ensure that the yeast cells remain at least 25 μm from the laser spot. Future autonomous manipulation will be able to maintain this separation with a faster reaction time, so the manipulation velocity can be increased.

CONCLUSION

Single cells in an agarose solution were patterned using the optically controlled thermocapillary flow surrounding a cavitation bubble in the solution. The agarose solution was gelled, and the patterned cells were able to grow in this 3D biocompatible scaffold. This opto-thermocapillary technique provides a unique, cell-friendly method of patterning for cell and tissue cultures and associated research. Although the parallel manipulation of multiple cells were not shown here, it is possible to achieve this in the future by projecting multiple laser spots on the substrate.

Several other key parameters of the experiment require further investigations to improve its performance. For example, a detailed temperature profile surrounding the bubble could show the cell an accurate safe distance, while the relation between laser pulse and the scale of the thermocapillary flow can give a better picture of this tool's resolution. Finally, current experiment used yeast cells in assembly tasks due to its fast growth. Mammalian cells should be tested by the same procedure in the future in order to apply this tool to broader applications.

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