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Flow manipulation and cell immobilization for biochemical applications using thermally responsive fluids

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A flow redirection and single cell immobilization method in a microfluidic chip is presented. Microheaters generated localized heating and induced poly (N-isopropylacrylamide) phase transition, creating a hydrogel that blocked a channel or immobilized a single cell. The heaters were activated in sets to redirect flow and exchange the fluid in which an immobilized cell was immersed. A yeast cell was immobilized in hydrogel and a 4′,6-diamidino-2-phenylindole (DAPI) fluorescent stain was introduced using flow redirection. DAPI diffused through the hydrogel and fluorescently labelled the yeast DNA, demonstrating *in situ* single cell biochemistry by means of immobilization and fluid exchange. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4768905]

The ability to control microfluidic flow is central to nearly all lab-on-a-chip processes. Recent developments in microfluidics either include microchannel based flow control in which microvalves are used to control the passage of fluid,[1] or are based on discrete droplet translocation in which electric fields or thermal gradients are used to determine the droplet path.[2,3] Reconfigurable microfluidic systems have certain advantages, including the ability to adapt downstream fluid processes such as sorting to upstream conditions and events. This is especially relevant for work with individual biomolecules and high throughput cell sorting.[4] Additionally, reconfigurable microfluidic systems allow for rerouting flows around defective areas for high device yield or lifetime and for increasing the device versatility as a single chip design can have a variety of applications.

Microvalves often form the basis of flow control systems and use magnetic, electric, piezoelectric, and pneumatic actuation methods.[5] Many of these designs require complicated fabrication steps and can have large complex structures that limit the scalability or feasability of complex microfluidic systems. Recent work has shown how phase transition of stimuli-responsive hydrogels can be used to actuate a simple valve design.[6] Beebe *et al*. demonstrated pH actuated hydrogel valves.[7] Phase transition of thermosensitive poly(N-isopropylacrylamide) (PNIPAAm) using a heater element was demonstrated by Richter *et al*.[8] Phase transition was also achieved by using light actuation by Chen *et al*.[9] Electric heating has shown a microflow response time of less than 33 ms.[11] Previous work[10] showed the use of microheaters to induce a significant shift in the viscosity of thermosensitive hydrogel to block microchannel flow and deflect a membrane, stopping flow in another microchannel. Additionally, Yu *et al*.[12] demonstrated thermally actuated valves based on porous polymer monoliths with PNIPAAm. Krishnan and Erickson[13] showed how reconfigurable optically actuated hydrogel formation can be used to dynamically create highly viscous areas and thus redirect flow with a response time of ∼2 s. This process can be used to embed individual biomolecules in hydrogel and suppress diffusion as also demonstrated

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by others.[15,16] Fiddes *et al.*[14] demonstrated the use of hydrogels to transport immobilized biomolecules in a digital microfluidic system. While the design of Krishnan and Erickson is highly flexible, it requires the use of an optical system and absorption layer to generate a geometric pattern to redirect flow.

This paper describes the use of an array of gold microheaters positioned in a single layer polydimethylsiloxane (PDMS) microfluidic network to dynamically control microchannel flow of PNIPAAm solution. Heat generation and thus PNIPAAm phase transition were localized as the microheaters were actuated using pulse width modulation (PWM) of an applied electric potential. Additionally, hydrogel was used to embed and immobilise individual cells, exchange the fluid parts of the microfluidic system in order to expose the cells to particular reagents to carry out an *in situ* biochemical process. The PDMS microchannel network and the microheater array are shown in Figure 1.

The microchannels were fabricated using a patterned mould on a silicon wafer to define PDMS microchannels, as described by DeBusschere *et al.*[17] and based on previous work.[10] A 25×75 mm glass microscope slide served as the remaining wall of the microchannel system as well as the substrate for the microheater array. The gold layer had a thickness of 200 nm and was deposited and patterned using E-beam evaporation and photoresist lift-off.[21] The gold was patterned to function as connecting electrical conductors as well as the microheaters.

It was crucial that the microheater array was aligned with an accuracy of $\sim 20\,\mu$m with the PDMS microchannel network for good heat localization. The PDMS and glass lid were treated with plasma to activate the surface and alignment was carried out by mounting the microscope slide onto the condenser lens of an inverted microscope (TE-2000 Nikon Instruments). While imaging with a $4\times$ objective, the *x, y* motorized stage aligned the microchannels to the heaters and the condenser lens was lowered for the glass substrate to contact the PDMS and seal the microchannels.

Local phase transition of 10% w/w PNIPAAm solution in the microchannels was achieved by applying a 7 V potential through a H-bridge that received a PWM input at 500 Hz which was modulated using a USB controller (Arduino Mega 2650) and a MATLAB (Mathworks) GUI. The duty cycle of the PWM input was calibrated for each microheater to account for differences in heater resistances (25 Ω to 52 Ω) due to varying lengths of on-chip connections and slight fabrication inconsistencies, as well as for different flow conditions during device operation. Additionally, thermal cross-talk between heaters required decreasing the PWM input significantly when multiple heaters were activated simultaneously. This allowed confining the areas of cross-linked PNIPAAm to the microheaters, allowing the fluid in other areas to flow freely.

By activating the heaters in sets, it was possible to redirect the flow and exchange the fluid in the central area. Figure 2 demonstrates how the flow direction in the central microchannel area was changed from a stable horizontal flow to a stable vertical flow with a 3 s response time, using only PNIPAAm phase transition. Constant pressures were applied to the inlets to

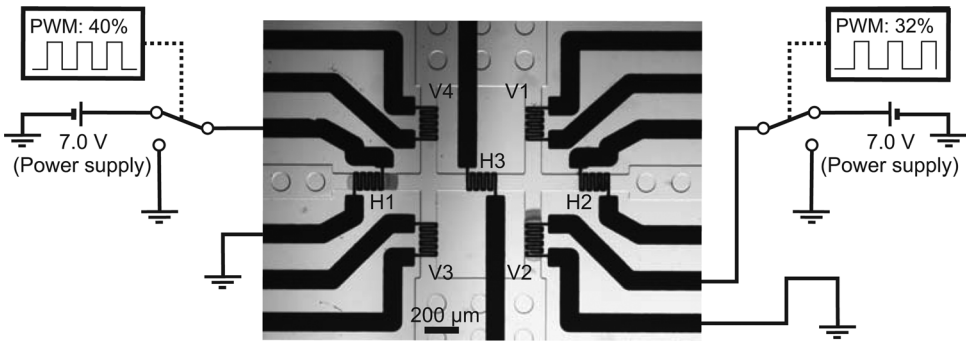

FIG. 1. A sketch of the electrical circuit and a microscope image of the gold microheaters and the PDMS microchannels. The power to the heaters was modulated with a PWM input through a H-bridge. For clarity, the electrical circuit for only the two heaters with gelled PNIPAAm is shown (H1 and V2). There are four heaters (V1-V4) in the "vertical channels" and three heaters (H1-H3) in the "horizontal" channel.

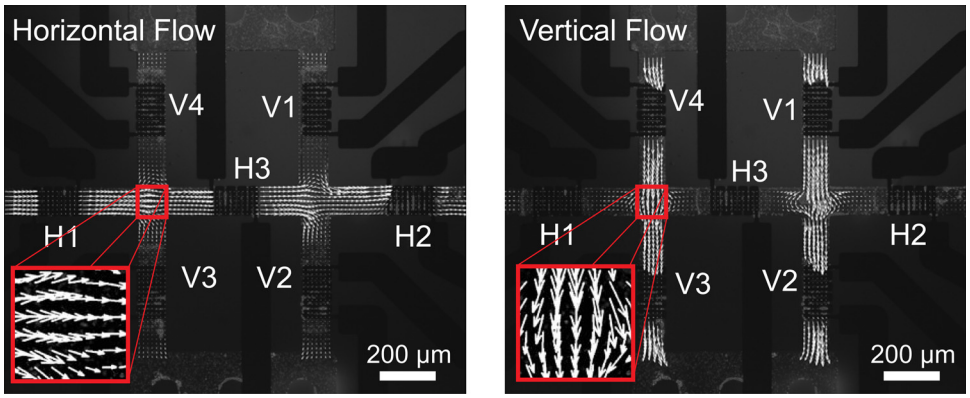

FIG. 2. Switching between fluid from the horizontal and the vertical channel using hydrogel activation and flow redirection with a response time of 3 s. A pressure of 25 mbar was applied to the inlet of the horizontal channel and a pressure of 20 mbar to the vertical channel. The flow field was determined using particle image velocimetry, in which the displacement of fluorescent seed particles was determined from image pairs generated by laser pulse exposure. Processing was carried out with DAVIS software (LaVision).

the horizontal channel and to the vertical channels. Activating heaters V1-4 (Figure 2, left) resulted in flow in the horizontal channel only. Likewise, activating heaters H1 and H2 allowed for flow in the vertical channel only. In this sequence, the fluid in the central microchannel area from one inlet was exchanged with fluid from the other inlet. Additionally, by activating heater H3, a particle could be immobilised during the exchange of fluid as shown in Figure 3 (top).

Particle immobilisation in hydrogel and fluid exchange in the central area of the microfluidic network were used to carry out an *in situ* biochemical process in which a yeast cell injected through one inlet was stained *in situ* with a 4′,6-diamidino-2-phenylindole (DAPI) solution (Invitrogen), which attached to the DNA of the yeast cell.[18] A solution of yeast cells with a

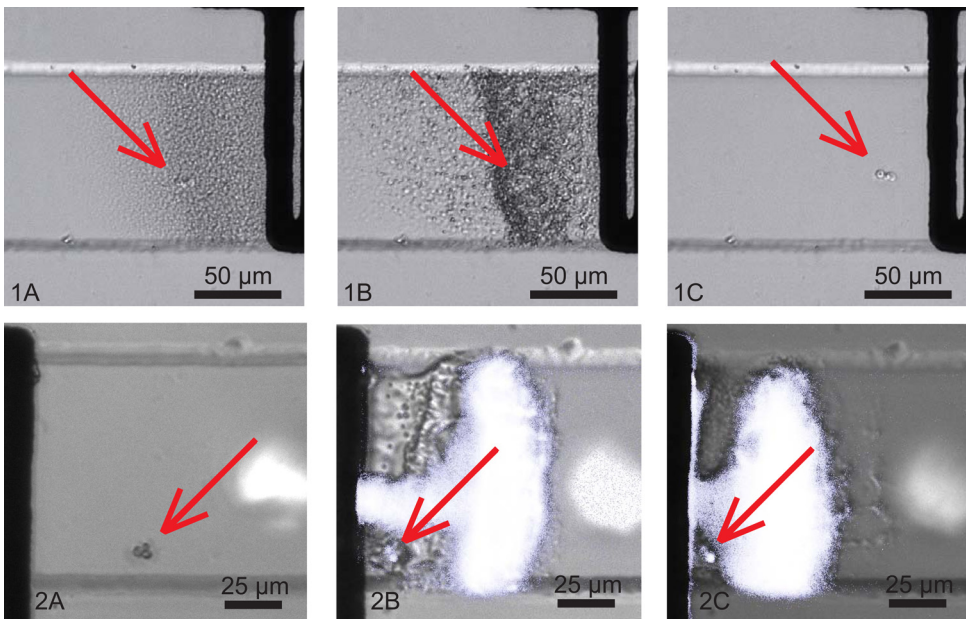

FIG. 3. A series of microscope images near heater H3 showing: (1a)-(1c) A single yeast cell captured by local PNIPAAm phase transition and immobilized for 5 min before being released. (2a) A single yeast cell was identified for capture by embedding in hydrogel. (2b) The cell as well as the hydrogel displayed fluorescence while embedded due to the introduction of DAPI in the surrounding region. (2c) The diffusion of DAPI towards the cell as the heating power of H3 is reduced after 15 min, showing a DAPI stained yeast cell immobilized.

concentration of 5×10^7 cells/ml suspended in a 10% w/w PNIPAAm solution was injected through the horizontal channel. A solution of 2 μg/l DAPI in a 10% w/w PNIPAAm solution was injected through the vertical channel. A single yeast cell was identified and captured near the central heater, and by deactivating the heaters in the vertical channel, DAPI solution was introduced in the microchannels around the hydrogel. After immobilising the cell for 15 min, the heater was deactivated, releasing the cell in the DAPI solution. This process is shown in Figure 3 (bottom). The sequence of the heater activation and deactivation in order to immobilize the cell and exchange the fluid is outlined in the supplementary material.[21]

Eriksen *et al.*[15] demonstrated the diffusion of protease K in the porous hydrogel matrix,[19] and it was therefore expected that DAPI fluorescent stain (molecular weight of 350 kDa, Ref. 20) would also diffuse. DAPI diffusion is shown in Figure 3(2b) in which the yeast cell shows fluorescence while embedded in the hydrogel. The yeast cell was released by deactivating the central heater and activating all the others to suppress unwanted flow in the microchannel. As a result, the single cell was fully immersed in the DAPI solution. Immobilization of a single cell allows for selection of a cell that exhibits a certain trait and introduction of a new fluid while maintaining the cell position in the field of view of the microscope such that a biochemical response can be imaged continuously.

In summary, a microfluidic chip capable of local heating was used to induce phase transition of PNIPAAm to hydrogel, blocking microchannel flow, and thereby allowing for reconfigurable flow. Additionally, the hydrogel was used to embed and immobilise a single yeast cell. DAPI fluorescent stain was introduced using flow redirection, and it stained the immobilized cell, showing diffusion into the hydrogel. The versatile design of this microfluidic chip permits flow redirection, and is suitable to carry out *in situ* biochemical reactions on individual cells, demonstrating the potential of this technology for forming large-scale reconfigurable microfluidic networks for biochemical applications.

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