Thermo-sensitive Microgels as in-situ Sensor for Temperature Measurement in Optoelectronic Tweezers

Hsan-yin Hsu[1], Steffen Vogler[2], Arash Jamshidi[1], Justin Valley[1], Shao Ning Pei[1], Sophie Pautot[2], and Ming C Wu[1]
[1] Univeristy of California – Berkeley, USA
[2]DFG-Center for Regenerative Therapies Dresden, Germany

ABSTRACT

We report on the application of thermo-sensitive microgels as *in situ* temperature sensor for phototransistor-based optoelectronic tweezers (Ph-OET) device. The thermo-sensitive microgels are cross-linked polymeric particles that swell or shrink reversibly in response to changes in the surrounding temperature. The technique has an accuracy of 0.054ºC and a spatial resolution of 25µm. Temperature rise in Ph-OET is measured under various operating conditions, and the maximum temperature increase is measured at 2.6ºC. The results show cell damage can be prevented with adequate heat sink. When physiological temperature is required, the applied bias should be kept low ($10V_{pp}$), or smaller optical patterns should be used. The technique demonstrated here can be extended to other microfluidic devices.

INTRODUCTION

Dynamic manipulation of biological cells and micro/nano-particles has many applications in fundamental researches. Previously, we have proposed a massively parallel optical manipulation technique called Optoelectronic Tweezers (OET) [1]. Based on light-induced dielectrophoresis (DEP), OET has 100,000x lower optical intensity requirement than optical tweezers. Parallel manipulation has been demonstrated using direct optical images from digital projectors. OET has been used to manipulate various cell types, including yeast, bacteria, and mammalian cells. Phototransistor-based OET [2] (Ph-OET) allows OET operation in media with physiological salinity, a more natural environment for cells. Using Ph-OET, sorting of adherent neurons for cell replacement therapy has been proposed [3].

In all cell manipulation devices, it is important to control the buffer temperature to within a couple of degrees to avoid cell damage. Hyperthermic stress or cell damage can be induced by either a short, sharp increase or a persistent moderate increase of temperature above physiology condition [4]. Some applications such as cell culturing require precise temperature maintenance. DEP is known to induce problematic temperature rise due to joule heating [5]. Since OET is based on light-induced DEP, it is subject to similar heating. Joule heating is more pronounced in high conductivity solution. Ph-OET, which works in high conductivity solution, generates more heat than conventional OET. Therefore, to avoid cell damage, it is important to measure, and ultimately controls, the temperature cells experience inside the Ph-OET chamber.

Existing temperature measurement techniques for microfluidic devices are inadequate for Ph-OET device. The most commonly used method is temperature-dependent fluorescent microscopy [6], which uses fluorescent dyes whose intensities vary with temperature. However, the technique has limited accuracy (0.85ºC) due to low sensitivity. In addition, the fluorescent signal is integrated over the height of the chamber rather than measuring the local temperature around the cell. The fluorescence may also interfere with other fluorescent markers used in cell assays. Here, we demonstrate a novel *in situ* temperature sensor using thermo-sensitive microgel beads. It has higher accuracy and better resolution in the direction perpendicular to the image plane. By changing its water content, the cross-linked gel can dramatically alter its size in response to local temperature. Standard image processing software allows accurate determination of the relative size of the microgels beyond diffraction limit. The temperature has been measured with an accuracy of 0.054ºC and a spatial resolution of 25µm. Using these thermo-sensitive microgels, we have successfully characterize temperatures of Ph-OET under various operating conditions. Our results show that precise temperature control in Ph-OET device can be accomplished with proper thermal management.

EXPERIMENTAL SETUP

The Ph-OET device consisted of a two-dimensional array of pixelated phototransistors, with a pixel size of 10µm x 10µm. It was fabricated on 6-inch silicon

978-1-4244-5764-9/10/$26.00 ©2010 IEEE

wafers. The detailed fabrication has been reported elsewhere [2]. The device assembly (Fig. 1a) consisted of sandwiched layers of 1mm thick ITO-coated glass, 100µm thick liquid media, and the Ph-OET substrate. Metal films with 10nm titanium and 150nm gold were deposited on the backside of Ph-OET substrate for electrical contact. Phosphate-buffered saline (PBS) containing the microgels were introduced as the media. The chip was mechanically pressed against an aluminum stage, whose temperature is controlled to within 0.1ºC accuracy by a thermoelectric heater. The aluminum stage served as a heat sink for controlling the device's ambient temperature.

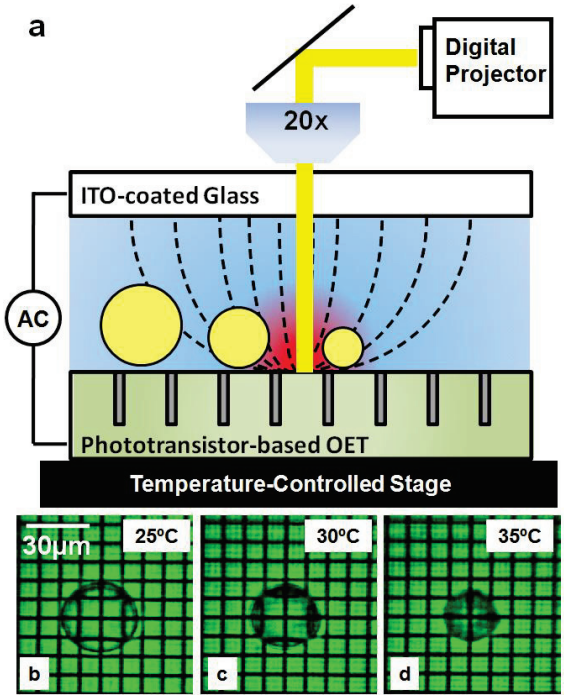

Fig 1. (a) Schematic of Ph-OET device and the experimental set-up. The light pattern activates the phototransistors and induces a local electric field. The thermo-sensitive microgels in the media respond to temperature rise by shrinking **(b-d) Images of Thermo-sensitive microgel at various temperatures.** As temperature increase, the microgels shrink in size.

To operate the device, an AC voltage was applied between the Ph-OET substrate and top ITO electrode. Light patterns from a digital micromirror device (DMD) projector were imaged onto the device through a 20x objective lens. The final light intensity is 1 W/cm^2. The light intensity is sufficient to turn on the phototransistors. The illuminated phototransistors pixels generate electric fields inside the media and interact with nearby cells through DEP. The device was monitored under bright field illumination, and the data was recorded with a CCD camera.

The microgels were prepared using capillary microfluidic devices developed to fabricate monodisperse single and multiple emulsions [7]. This method allows for the uniform polymerization of the microgel regardless of the particle size, which greatly improves the sensitivity of the switching properties. The polymer chains forming these microgels contain both hydrophilic amide groups and hydrophobic isopropyl groups. When dispersed in water at low temperatures, generally below 32ºC, the amide groups of the microgels interact strongly with water through hydrogen bonding and these strong interactions force water into the microgels, causing them to swell. However, at higher temperatures, the hydrogen bonds between water and the amide groups are disrupted, making water a poor solvent. As a result, water is expelled from the microgels, the polymer network collapses, and the particles shrink dramatically (Fig. 1b-d).

For this work, we have used microgel particles with size near 25µm in diameters, similar to that of mammalian cells. These particles are ideal thermal probe for devices designed to handle living cells. They are polyelectrolytes with high water content; their size, index of refraction and overall charge are comparable to those of cells. They can thus be considered as a model cell, with the added benefit that they provide a readout of the surrounding temperature. The particles can be trapped and transported with Ph-OET, while providing temperature reading (Fig. 2). Similar to mammalian cells, the thermo-sensitive microgels experience a negative DEP force in PBS.

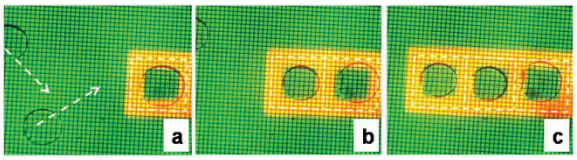

Fig 2. Trapping of thermo-sensitive microgels with Ph-OET. Square optical patterns were used to trap microgel particles. The applied voltage is 10Vpp at 1MHz frequency.

RESULTS

Thermo-sensitive Microgels Characterization

The normalized radius of the microgels as a function of temperature are measured and shown in Fig. 3. The measurement is performed by taking images of microgels at various stage temperatures. The radius is normalized to that at 23ºC. The radius vs. temperature curve is roughly linear between 32-35ºC, providing an ideal range for temperature readout. A 25µm microgel has a sensitivity of roughly 1µm change in diameter per

degree Celsius change in temperature. Using this method, the temperature accuracy is determined by the resolution of the optical system. Typical microscope with low NA (0.4; 550nm wavelength) objective has a resolving power of 700nm. This gives a temperature readout accuracy of 0.7 ºC.

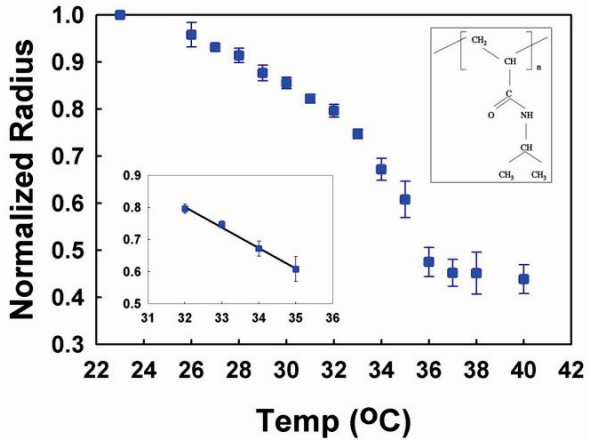

Fig 3. Radius of poly(N-isopropylacrylamide) microgels versus temperature. The radius is normalized to their size at 23ºC.

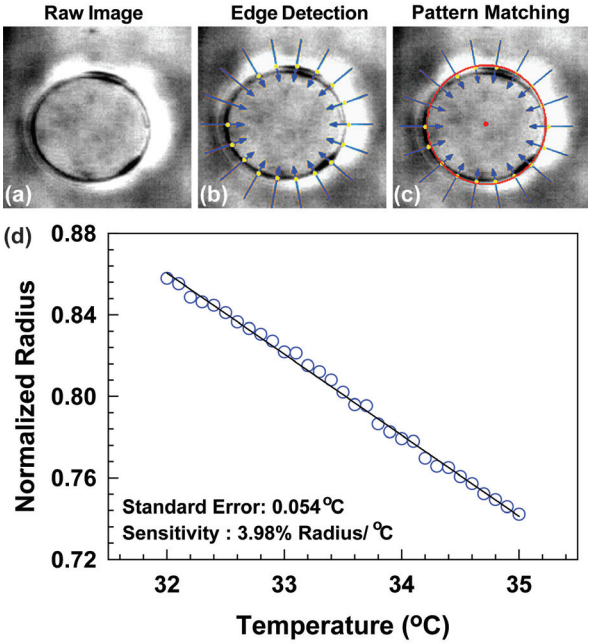

Fig 4. (a-c) Pattern matching process. (d) Normalized microgels radius as a function of temperature. The size of the microgel is measured with pattern matching.

Pattern matching technique allows the determination of microgels' sizes beyond diffraction limit. We use LabView program to find the radius of the particles (Fig. 4a-c). The program starts out with finding multiple points on the microgel. Based on the points found, a perfect circle is fitted representing the microgel edge. The radius of the circle was then recorded. Using this program, a more precise temperature versus microgels radius were measured for every 0.1ºC from 32ºC to 35ºC, and the results is shown in Fig. 4d. The temperature and radius was fitted as a linear relation. The sensitivity is calculated to be 3.98% radius/ºC, and the standard error is 0.054ºC.

Ph-OET Temperature Characterization

The temperature rise in Ph-OET chamber was measured as a function of applied voltage with and without illumination (Fig. 5). In the absence of light, the temperature rise is less than 0.2ºC at $10V_{pp}$ and 1.6ºC at $20V_{pp}$. When the projector is fully turned on, additional temperature increases of 0.2ºC and 1ºC were observed at $10V_{pp}$ and $20V_{pp}$, respectively.

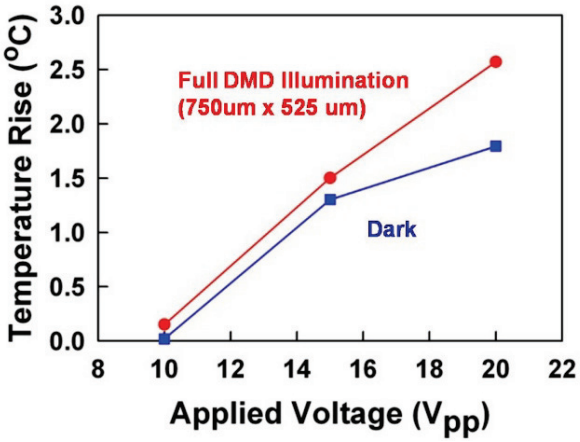

Fig 5. Temperature rise vs. electrical bias with and without illumination. The applied voltage was $20V_{pp}$ at 1MHz.

Ideally there should be no temperature rise in the absence of light as the phototransistor is in the cutoff mode and all currents are blocked by the phototransistor. However, because the phototransistor has an asymmetric doping profile (N^+-P-N^-), there is some leakage current in the reverse cutoff mode, i.e, when the voltage applied to the substrate is negative, due to partial punch-through. This temperature increase is uniform across the Ph-OET chip, which can be compensated by external cooling. The temperature increase from light illumination, on the other hand, is local and caused by activation of the phototransistors. The temperature rise is confined to the illuminated area. Since this cannot be compensated externally, this temperature rise should be minimized. The light-induced temperature rise is dependent on the light pattern and total illumination area.

To better characterize the temperature of Ph-OET under typical operating conditions, we performed a detailed measurement of the microgel temperature with various

light patterns. The bias voltage is kept constant at $20V_{pp}$. Figure 6 shows the temperature rise for square patterns. The temperature rise due to light is insignificant for patterns smaller than 140µm x 140µm. For larger patterns, modest temperature rise was observed: 0.5ºC for 280µm x 280µm and 0.7ºC for 525µm x 525µm.

Figure 7 shows the temperature rise versus the number of cell traps. Each trap consists of a square ring with 25µm line width and 30µm x 30µm dark area (see inset of Fig. 7). The temperature rise is similar to the dark level for array size smaller than 7 x 7. A modest temperature rise of 0.2ºC above dark level was observed for 8 x 8 arrays.

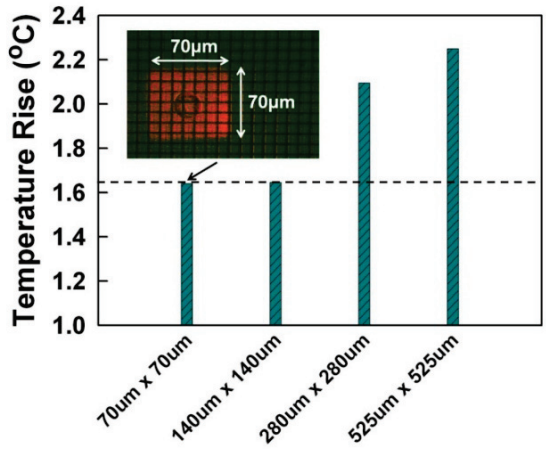

Fig 6. Measured temperature rise with square pattern of various sizes under $20V_{pp}$ voltage bias. The dotted line shows the temperature rise without illumination.

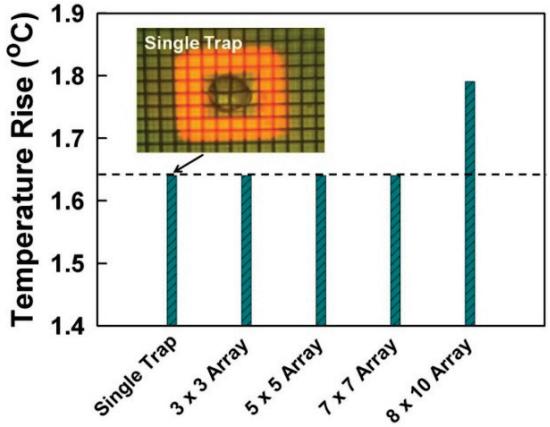

Fig 7. Measured temperature rise with different numbers of single particle trap under $20V_{pp}$ bias voltage. The dotted line shows the temperature rise without illumination.

CONCLUSION

The thermo-sensitive microgels allow *in situ* measurement of microfluidic temperature with high sensitivity (0.054ºC) and high resolution (25µm), while being a good cell model. The microgel itself can be used as a model cell as it has similar size and properties. We applied this technique to measure the temperature of phototransistor-based optoelectronic tweezers (Ph-OET) device under typical operation conditions. The temperature rise due to voltage bias alone was found to be 0.2ºC for $10V_{pp}$ bias and 1.6ºC for $20V_{pp}$. Light illumination does not introduce significant temperature rise for trap array size smaller than 7x7. Modest temperature rise (0.2ºC) was observed for large array (8x8). The technique described in this paper can also be applied to other cell handling microfluidic devices.

REFERENCE

[1] P.Y. Chiou, A.T. Ohta, and M.C. Wu, "Massively parallel manipulation of single cells and microparticles using optical images," *Nature*, vol. 436, 2005, pp. 370-372.

[2] H.Y. Hsu, A.T. Ohta, P.Y. Chiou, A. Jamshidi, S.L. Neale, and M.C. Wu, "Phototransistor-based optoelectronic tweezers for dynamic cell manipulation in cell culture media," *Lab Chip*, 2009.

[3] H.Y. Hsu, S. Pautot, H. Lee, K. Yu, S.L. Neale, A.T. Ohta, A. Jamshidi, J. Valley, E. Isocaff, and M.C. Wu, "Sorting of differentiated neurons using phototransistor-based optoelectronic tweezers for cell replacement therapy for neurodegenerative diseases," T*RANSDUCERS 2009* p. 1598.

[4] J. Vanrijn, J. Vandenberg, J. Souren, R. Vanwijk, and H. Joenje, "Hepatoma-Cells Adapted To Proliferate Under Normally Lethal Hyperthermic Stress Conditions Show Rapid Decay Of Thermoresistance And Heat-Shock Protein-Synthesis When Returned To 37-Degrees-C," *Int. J. Hyperthermia*, vol. 11, Oct. 1995, pp. 697-708.

[5] U. Seger-Sauli, M. Panayiotou, S. Schnydrig, M. Jordan, and P. Renaud, "Temperature measurements in microfluidic systems: Heat dissipation of negative dielectrophoresis barriers," *ELECTROPHORESIS*, vol. 26, 2005, pp. 2239-2246.

[6] D. Ross, M. Gaitan, and L.E. Locascio, "Temperature Measurement in Microfluidic Systems Using a Temperature-Dependent Fluorescent Dye," *Analytical Chemistry*, vol. 73, 2001, pp. 4117-4123.

[7] " L. Y. Chu, A. S. Utada, R. K. Shah, J. W. Kim and D. A. Weitz, Angew. Chem., Int. Ed., 2007, 46, 8970–8974"