Temperature measurement in microfluidic chips using photobleaching of a fluorescent thin film

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A method for the whole chip temperature measurement is developed and presented here. This method includes two major contributions: (i) a specially developed measurement model illustrating the relationship between the photobleaching speed of a fluorescent dye and its temperature and (ii) an introduction of a thin polydimethylsiloxane film with rhodamine B homogeneously saturated aiming for significantly reducing fluorescent dyes' absorption to and diffusion into polymer-made channel walls. The developed method is validated by comparing the experimentally measured temperature distribution in a microfluidic chip with the numerically predicted results. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2828717]

Temperature measurement is key to microfluidic chip applications involving heat transfer[1–5] and becomes more prominent for polymer-made chips[6] due to their low thermal conductivities. Many methods have been developed for on-chip temperature measurement,[7–11] among which the method[7] involving the mixing of a temperature-dependent fluorescent dye (rhodamine B) with the working liquid is very promising. Improvements[10,11] have been made to consider the nonuniformal background effects using two dyes. However, these methods have several limitations when applied to polymer-made chips. The major drawback is that fluorescent dye particles can quickly diffuse into polymer channel walls[12,13] causing significant measurement errors. Efforts have been made to reduce this effect,[13,14] however, either no significant reduction was found or the method was too complicated. In addition, these methods did not consider inevitable dye photobleaching.[15,16]

A method is developed utilizing the fact that most fluorescent dyes have a temperature-dependent photobleaching speed. This method has two major contributions. First, a 40 μm thick polydimethylsiloxane (PDMS) film with rhodamine B dye homogeneously saturated[17] is introduced into the chip to reduce the diffusion problems and realize the whole chip temperature measurement. By monitoring the photobleaching speed of the fluorescent thin film, its temperature distribution can be obtained. It should be noted that the fluorescent particles in the thin film can diffuse back to the liquid slowly. However, it will not affect the temperature measurement much because the diffusion of dye particles from the thin film to fluid is much slower than the other direction according to experimental results. Second, a measurement model is developed to illustrate the relationship between the photobleaching speed of a fluorescent dye and its temperature. This relationship indicates that this method is independent on the concentration of the fluorescent dye and the background noise. The uneven excitation light intensity distribution is also considered.

The schematic model used to develop the above-mentioned relationship is shown in Fig. 1(a), where a 3 mm diameter excitation light is projected to a 25×76 mm^2 PDMS-rhodamineB film. The visual field is a 360×360 μm^2 square centered at the film and divided into 10 000 small cells denoted by $S_{i,j_{(1\leqslant i,j\leqslant100)}}$. Uniform properties are assumed for each cell.

Background light exists because of light dispersion, reflection, and refraction. Therefore, the recorded fluorescent intensity for each cell can be written as

$$G_{i,j} = G_{i,j\_\text{rhodamine}} + G_{i,j\_\text{background}} \tag{1}$$

where $G_{i,j\_\text{rhodamine}}$ and $G_{i,j\_\text{background}}$ are the fluorescent intensity from the (i,j)th cell and its background, respectively. All the fluorescent intensity images were taken at the liquid/film interface. The error induced by the film thickness is discussed in the EPAPS material.[20] The fluorescent intensity is proportional to the dye concentration ρ and excitation light intensity I such as

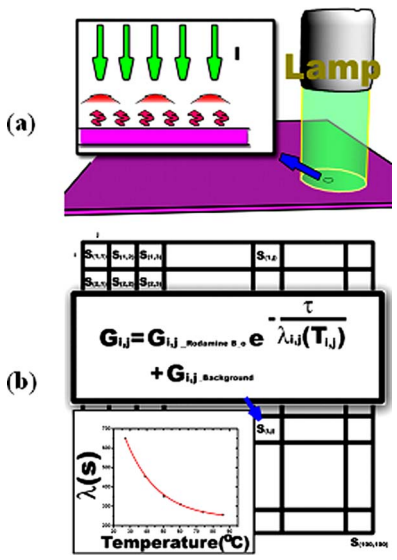

FIG. 1. (Color online) (a) Schematic of the measurement model and (b) illustration of the visual domain for exponential curve fitting.

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$$G_{i,j\_\text{rhodamine}} = k_1(T_{i,j})\rho_{i,j}I_{i,j}, \tag{2}$$

where $k_1(T_{i,j})$ is the coefficient considering the temperature dependence. The photobleaching speed is proportional to the dye concentration and excitation light intensity for a given temperature

$$d\rho_{i,j}/d\tau = -k_2(T_{i,j})I_{i,j}\rho_{i,j}, \tag{3}$$

where τ is time and $k_2(T_{i,j})$ is a coefficient considering the temperature dependence of the dye photobleaching speed. The temperature change due to light exposure is negligible because of the high transparency of PDMS and glass. Therefore, the temperature in each cell is considered constant over the photobleaching period and so does $k_2(T_{i,j})$. Integrating Eq. (3) with respect to time yields

$$\rho_{i,j} = \rho_0 e^{-k_2(T_{i,j})I_{i,j}\tau}, \tag{4}$$

where ρ_0 is the original dye concentration. Substituting Eq. (4) into Eq. (2) and considering $G_{i,j\_\text{rhodamine}\_0} = k_1(T_{i,j})I_{i,j}\rho_0$ at $\tau = 0$, the fluorescent intensity from the (i,j)th cell at time τ is

$$G_{i,j\_\text{rhodamine}} = G_{i,j\_\text{rhodamine}\_0} e^{-k_2(T_{i,j})I_{i,j}\tau}. \tag{5}$$

For a given cell, the captured background fluorescent intensity is a function of the excitation light intensity, location, dye concentration, and temperature distribution in the background region. For simplicity, this intensity is assumed constant for a given system and will be determined through curve fitting the fluorescent intensity values measured during a photobleaching process (see EPAPS material). Substituting Eq. (5) into Eq. (1) gives rise to

$$G_{i,j} = G_{i,j\_\text{rhodamine}\_0} e^{-k_2(T_{i,j})I_{i,j}\tau} + G_{i,j\_\text{background}}. \tag{6}$$

The rate change of the fluorescent intensity, which shows the photobleaching speed, can be obtained by taking derivative of Eq. (6) with respect to time,

$$\frac{dG_{i,j}}{d\tau} = -\frac{1}{\lambda(T_{i,j})} G_{i,j\_\text{rhodamine}\_0} e^{-[\tau/\lambda(T_{i,j})]}, \tag{7}$$

where $\lambda(T_{i,j}) = 1/[k_2(T_{i,j})I_{i,j}]$ is the characteristic time scale of photobleaching as a function of temperature and location. For each cell, $\lambda(T)$ is a function of temperature only. If a series of fluorescent intensity images are captured for this location at a temperature T_1 the value of $\lambda(T_1)$ can be determined for this location through curve fitting the recorded fluorescent intensity values with respect to time. Using this way, a calibration curve $\lambda(T_{i,j\_k})$ can also be obtained, where T_{i,j_k} is the kth temperature at the (i,j)th cell. This calibration curve allow the temperature at a certain location to be determined once its $\lambda(T_{i,j})$ is known.

From Eq. (7), it can be seen that, theoretically, this method is independent on the dye concentration and its background fluorescence, which are all considered through curve fitting the experimentally captured fluorescent intensity images. In this study, the microfluidic chip consists of a 2.12 mm thick PDMS substrate with a 100 μm $\times$ 20 μm $\times$ 5 cm microchannel, a 40 μm thick PDMS film saturated with rhodamine B, and a 1 mm thick glass substrate on which the thin PDMS-rhodamineB film is spin coated, as shown in Fig. 2(a). The PDMS channel was fabricated using the standard soft lithography techniques.[18] The PDMS-rhodamineB film was obtained by soaking it into a 10 mM rhodamine B solution (Acros Organics) for 2 days. Experiments showed that a PDMS thin film could be saturated after 1–2 days.

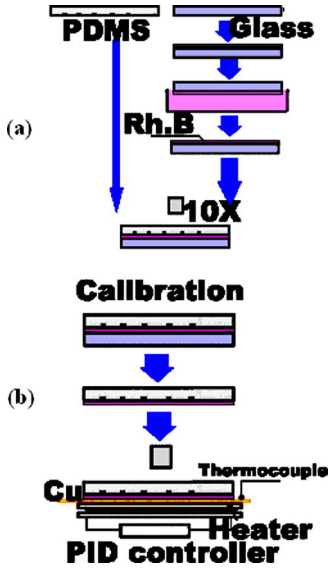

FIG. 2. (Color online) Flow chart for (a) fabrication and (b) calibration.

Transversal temperature gradients were established in the PDMS-rhodamineB film through heat transfer from the liquid (i.e., 20 mM KCl solution) where Joule heating is generated using a 500 V/cm electrical field to the rest of the film. After the temperature distribution reaches steady state, imaging of the fluorescent film was performed using a fluorescence microscope system equipped with a mercury arc lamp (100 W) and an appropriate filter (excitation of 510–550 nm).[18] Experiments showed that the variation of the light intensity is $<1\%$ over a 10 min period, which is negligible. For each location, 301 fluorescent images were taken with a 2.5 s time interval and normalized by the initial fluorescent image. Equation (6) can be reduced to

$$G'_{i,j} = A_{i,j}e^{-\tau/\lambda_{i,j}} + B_{i,j}, \tag{8}$$

where $A_{i,j} = G_{i,j\_\text{rhodamine}\_0}/G_{i,j\_0}$ and $B_{i,j} = G_{i,j\_\text{background}}/G_{i,j\_0}$ which are both functions of location and constant for each cell. Equation (8) was used to curve fit the experimentally measured fluorescent intensity and time relationship for each location, which allows the value of $\lambda(T_{i,j})$ to be determined. Similar procedures were used to perform calibration of $\lambda(T_{i,j\_k})$ using a special design, as shown in Fig. 2(b). Briefly, a PDMS/PDMS-rhodamineB chip is pressed tightly onto a copper plate whose temperature was kept stable by a heater, controlled by a temperature controller (TC-24-25 RS232, TE technology) and monitored using a thermocouple. The calibration curve for $\lambda(T_{i,j\_k})$ was obtained at the temperatures of 27.1, 39.2, 50.5, 60.5, 74.0, and 86.0 °C with five times of photobleaching for each temperature. The temperature uncertainty is around 0.405 °C.

To validate the measurement method, a previously reported three-dimensional numerical model[19] was employed with slight modifications. Figures 3(a) and 3(b) show the numerically predicted and experimentally measured temperature distribution within the visual field, respectively. The total measurement time is 12.5 min allowing 301 images to be taken. One can see that the temperature is almost uniform across the channel at a temperature around 73 °C, which is

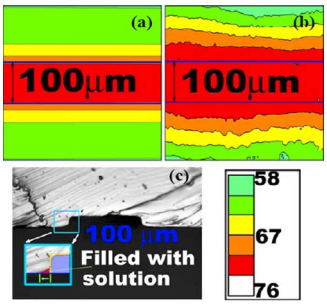

FIG. 3. (Color online) Comparison between numerically predicted and experimentally measured temperature distribution at the interface between the liquid and PDMS-rhodamineB film within the visual field. (a) Numerical results, (b) experimental results, and (c) image of the cross section of the microchannel.

because Joule heating is uniformly generated in a straight microcahnnel. The temperature decreases from the channel transversely giving a temperature range from 73 to 58 °C in the rest of the film, which is consistently observed in both numerical and experimental results. It can be seen that the measured temperatures outside of the channel are higher than that numerically predicted with a maximum difference of 6 °C. This is mainly because a small gap exists between the two channel substrates due to fabrication, as highlighted in Fig. 3, which is not considered in numerical simulation. During experiments, this gap is filled with the working liquid where Joule heating is generated, which increases its temperature as observed experimentally.

There are several potential approaches which may improve the accuracy of this technique. First, a thinner PDMS-rhodamineB film will reduce the measurement erros. The techniques for fabricating a super thin PDMS film are under development. Second, increasing the image capturing speed will shorten the time required for photobleaching minimizing the temperature variation due to exposure to the excitation light source. In addition, in order to get a transient temperature distribution, the photobleaching time should be controlled under 0.1 s.

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[1]L. Liu, S. Peng, W. Wen, and P. Sheng, Appl. Phys. Lett. **91**, 093513 (2007).
[2]S. K. Y. Tang, B. T. Mayers, D. V. Vezenov, and G. M. Whitesides, Appl. Phys. Lett. **88**, 061112 (2006).
[3]J. Pawliszyn and J. Q. Wu, J. Microcolumn Sep. **5**, 397 (2001).
[4]B. Kates and C. L. Ren, Electrophoresis **27**, 1967 (2006).
[5]D. Ross and L. E. Locascio, Anal. Chem. **74**, 2556 (2002).
[6]G. Q. Hu, Q. Xiang, R. Fu, B. Xu, R. Venditti, and D. Q. Li, Anal. Chim. Acta **557**, 146 (2006).
[7]D. Ross, M. Gaitan, and L. E. Locascio, Anal. Chem. **73**, 4117 (2001).
[8]H. Watzig, Chromatographia **33**, 445 (1992).
[9]A. M. Chaudharl, T. M. Woudenberg, M. Albin, and K. E. Goodson, J. Microelectromech. Syst. **7**, 345 (1998).
[10]J. Sakakibara and R. J. Adrian, Exp. Fluids **26**, 7 (1999).
[11]H. J. Kim, K. D. Kihm, and J. S. Allen, Int. J. Heat Mass Transfer **46**, 3967 (2003).
[12]M. W. Toepke and D. J. Beebe, Lab Chip **6**, 1484 (2006).
[13]G. T. Roman, T. Hlaus, K. J. Bass, T. G. Seelhammer, and C. T. Culbertson, Anal. Chem. **77**, 1414 (2005).
[14]D. Erickson, X. Liu, R. Venditti, D. Q. Li, and U. J. Krull, Anal. Chem. **77**, 4000 (2005).
[15]G. R. Wang, Lab Chip **5**, 450 (2005).
[16]Z. P. Huang and N. L. Thompson, Biophys. Chem. **47**, 241 (1993).
[17]V. Romano, A. D. Zweig, M. Frenz, and H. P. Weber, Appl. Phys. B: Photophys. Laser Chem. **49**, 527 (1989).
[18]J. Taylor, M.A.Sc. thesis, University of Waterloo, 2007.
[19]L. Gui and C. L. Ren, Anal. Chem. **78**, 6215 (2006).
[20]See EPAPS Document No. E-APPLAB-92-030801 for Appendix A: error analysis for a definite film thickness, and Appendix B: exponential fitting of experimentally measured fluorescent intensity of the thin film. This document can be reached through a direct link in the online articles HTML reference section or via the EPAPS home page (http://www.aip.org/pubservs.epaps.html).