Dynamic temperature measurement in microfluidic devices using thermochromic liquid crystals†

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Thermochromic liquid crystals (TLCs) are used to explore the temperature transients during thermal cycling for microchip-based polymerase chain reaction (PCR). By analyzing the reflected spectra of the TLCs over time, temperature *vs.* time trajectories were extracted and overshoots/undershoots were estimated. To our knowledge, this is the first report of TLC-based dynamic temperature measurements in a microfluidic device for all PCR temperature stages.

Introduction

Precise, localized temperature control is key in adapting many molecular biology techniques to lab-on-a-chip (LOC) devices. A common example is the polymerase chain reaction (PCR), a technique that employs thermal cycling to amplify genetic material. Temperature overshoots or undershoots (even of short duration) are known to have detrimental effects, such as reducing the activity level of the replicating enzyme (Taq) or causing errors to be made in the copying process.[1,2] These effects can lead to false positives or false negatives.

As described by Iles *et al.*,[3] the temperature characterization of microfluidic devices is challenging, since it is difficult to measure their temperature without significant perturbations. Recently, thermotropic (phase change) liquid crystals were used for thermal calibration in a micro-device,[4] but this method is limited to measuring only one specific temperature. To measure a range of temperatures, it has been found that thermochromic liquid crystals (TLCs) provide a compact and effective method at the microscale.[3,5–7] We make use of cholesteric TLCs, microencapsulated slurries of optically active mixtures of organic chemicals that react to changes in temperature by changing colour.[8]

Past use of TLCs has measured the temperature either by mapping the hue/colour (*e.g.* as measured by RGB signal values) to temperature or by mapping the wavelength of the peak of the reflected light to temperature. However, that has not been without challenges. Chaudhari *et al.*[5] went so far as to say that automated image analysis was impractical due to weak signals. They found that attempts to increase the concentration of the TLCs (in order to obtain a stronger signal) failed because it led to the white colour dominating the other colours. Although Noh *et al.*[6] were successful in mapping colour to temperature for steady state temperatures in a microchip PCR application, this success was limited, in part, by problems due to bubble formation at the higher temperature range (*i.e.* above 90 °C). Liu *et al.*[7] also used a hue-based method for measurement of the steady-state temperature of a 12 nL reaction PCR volume. Iles *et al.*[3] used the TLC method to measure the steady-state temperature of an organic synthesis reaction within a microfluidic reactor, and suggested the use of TLCs for dynamic applications. Though much has been done with TLCs, particularly to verify PCR chamber temperatures in the steady-state,[5–7] some barriers hinder their use in a dynamic mode. In the present work, we demonstrate a novel TLC-based method that overcomes these barriers and we apply it to tracking temperature transients during PCR.

There is an emphasis in the LOC community towards developing rapid diagnostics. In this context, realizing rapid PCRs within microfluidic devices, while still avoiding temperature overshoots and undershoots during transitions is important. The TLC colour change bandwidth can be customized, but there exists a trade-off between resolution (sensitivity) in measuring temperature and the range of temperatures that can be measured.[6] Since temperature fluctuations as small as 1–2 °C are enough to affect PCR, we choose to employ TLCs that change colour over a tight ~3 °C range to ensure sufficient resolution in measuring temperature. By analyzing the reflected spectra over time of TLCs placed inside the reaction chamber of the microchip as they undergo thermal cycling, the temperature *vs.* time trajectory is computed. This is, to our knowledge, the first time that TLCs have been used dynamically within microfluidic devices.

Materials and methods

The tri-layered glass–PDMS–glass PCR microchips (Fig. 1) used here were based on patterned metal films for heating and temperature sensing. Integrated microvalves are included for confining the contents of the reaction chamber, eliminating the bubble formation problems observed by Noh *et al*.[6]

We implemented a PD/PI controller that accurately controlled the chamber temperature (within ~1 °C) during the steady-state. However, during transitions, it is challenging to determine the temperature accurately, a common scenario in microfluidics.[3] Here, TLCs are used to further characterize the dynamic behaviour of the temperature within the reaction chamber at transitions between temperature stages to check for temperature overshoots and undershoots.

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† The HTML version of this article has been enhanced with colour images.

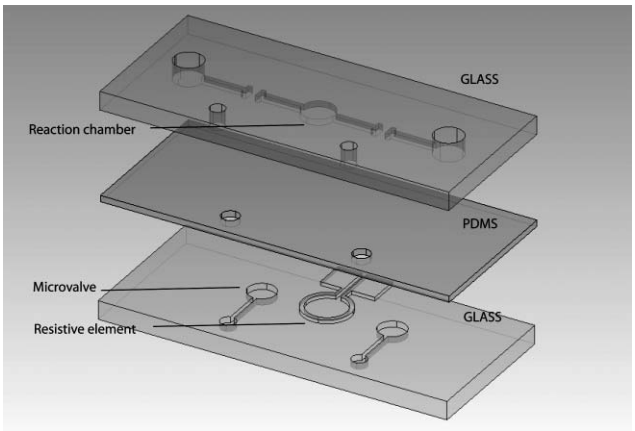

Fig. 1 Microchip architecture (48 mm × 14 mm) used for the genetic amplification. The reaction chamber is 90 μm deep and has a radius of 1.5 mm. Channel dimensions are 190 μm × 90 μm. The heater design and its placement with reference to other components were optimized to ensure temperature uniformity along the heater and in the reaction chamber.

Three sets of TLCs (R58C3W, R70C3W, R93C3W, Hallcrest, Glenview, IL, USA) were used, each custom-synthesized to change reflected colour with a bandwidth of ~3 °C around one of the typical desired chamber temperatures for each PCR stage. The reaction chamber is filled with a 1 : 2 dilution of the stock TLC suspension in water (we experimentally found that this concentration yielded the strongest colour signal without perturbing the TLC response), and the spectrum of the reflected light of the TLCs was observed every 100 ms (the actual temperature will vary on a time scale of seconds) using a spectrometer (Ocean Optics USB2000, Dunedin, FL, USA) in a setup similar to that used by Iles *et al.*[3] As suggested by the manufacturer, the TLCs were viewed at a perpendicular angle (using an optical fiber attached to the spectrometer) and with a black (non-reflective) background to ensure colour changes were prominent. Illumination was provided by a white LED and the ambient fluorescent lights. The transition to each temperature stage was tested individually (the procedure described later is repeated thrice) using the appropriate set of TLCs. The reflected spectra of the TLCs were recorded over time as the microchip underwent PCR temperature cycling (*i.e.* cycling through all three temperature stages repeatedly) and subsequently related to temperature (described later in this section). Because a narrow colour change bandwidth (~3 °C) was chosen, considerable intensity variations occur in a short temperature span, and this is sufficient to deduce the temperature of the TLCs to ~1 °C.

As seen by eye in reflected light under white light illumination, the TLC suspension has a milky white colour at room temperature. As it is heated, it changes colour successively (Table 1). The TLCs turn red as the temperature reaches the lowest temperature threshold, green at the second temperature threshold, and blue at the third threshold. When the temperature rises above the colour change range of the TLCs their colour returns to the original milky white.

Table 1 The colour change ranges of the custom-synthesized TLCs

TLC	Red	Green	Blue
R58C3W	58.0 °C	58.6 °C	60.8 °C
R70C3W	69.7 °C	70.6 °C	72.8 °C
R93C3W	92.4 °C	93.6 °C	95.8 °C

When the TLCs are white, the spectrometer detects a background spectrum consisting of strong peaks at the red (610 nm), green (535 nm), and blue (445 nm) wavelengths. We found that the background spectrum varied up to 10–20% between different runs due to slight variations in the positioning of the LED and optical fiber. Within a run, since the setup was not moved, the background spectrum generally remained constant. To account for background variations between runs, spectra collected over time during a run were divided by the background spectrum obtained at the beginning of each run. This results in a 3D plot of relative spectra (relative intensities *vs.* wavelength) *vs.* time. In some cases, the intensity of the battery-powered LED decreased slightly during the run, causing the background intensities to show a steady decrease that was easily subtracted. The background level is represented by 1 and increases in intensity have a relative intensity greater than 1.

To extract temperature *vs.* time data from these relative spectra *vs.* time plots, we make use of the blue peak in the spectrum, since it undergoes the largest intensity changes with temperature/colour, providing us with the largest sensitivity in temperature. Red and green peaks underwent smaller intensity changes and were hence not sufficiently sensitive.[8] The intensities over a bandwidth of ~15 nm around the blue peak (this corresponds roughly to its full width at half maximum) were averaged to reduce noise and subsequently plotted over time, yielding a plot of relative blue intensity over time. This plot was then normalized by dividing all relative intensities by the maximum relative blue intensity (B_{max}) reached throughout the run, yielding a plot of normalized blue intensity over time. Due to variations in illumination between different runs, this B_{max} value ranged from ~1.4–1.6. However, within a given run itself, the variation was less than 0.01. In the resulting normalized intensity *vs.* time plot (Fig. 2), the maximum value is now 1 (represents B_{max}), and different runs done with the same temperature set-points showed a variation of less than 0.01 in normalized intensity. Hence, with this normalization performed on the data (dividing by B_{max}), we can compare the data from different experimental runs.

Normalized blue intensity *vs.* time plots were obtained for runs done with different temperature set-points to obtain a correlation between the normalized blue intensity and temperature (Fig. 2a–c). Since the steady-state temperatures are well-calibrated, we calibrate our dynamic method to the steady-state method at points when the normalized blue intensity becomes constant with time. Hence, the value at which the normalized blue intensity settles is determined by the temperature set-point used (Table 2). We found that, as expected, B_{max} occurs approximately in the middle of the colour change range of the TLCs (*e.g.* ~72 °C, Fig. 2b). Hence, a given normalized blue intensity can be mapped to one of two different temperatures, one on the lower side of the colour change range and one on the higher side. To distinguish between the two, the normalized intensities of the red peak were plotted over time as well, following the same procedure that was applied to the blue peak (still normalizing to B_{max}). When the temperature is on the lower side of the TLC colour change range (*e.g.* 70 °C, Fig. 2a), there is an intensity increase in the red peak, and when the temperature

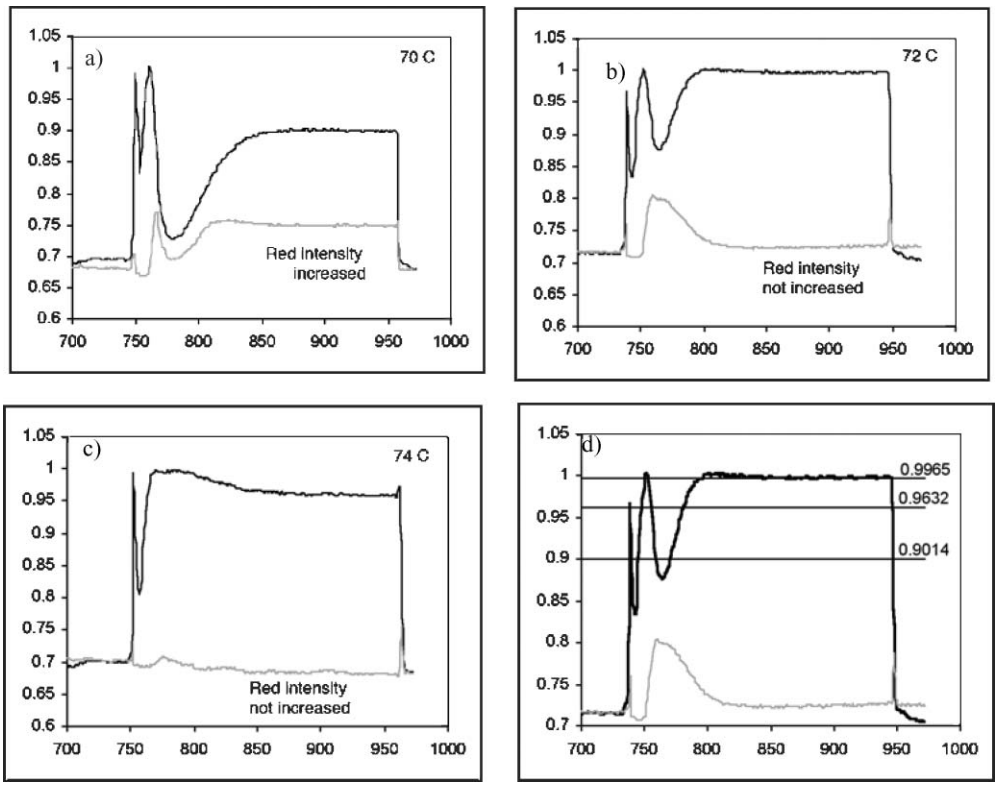

Fig. 2 Normalized intensity *vs.* time plots for controller set-points at (a) 70 °C, (b) 72 °C, and (c) 74 °C. The black lines represent the blue peak and the grey lines represent the red peak. Though data was collected over two PCR cycles (3 temperature stages each), only the 72 °C stage is shown here. In (d) the normalized intensity *vs.* time used to determine the temperature crossing points for the transition from the 60 °C to the 72 °C stage is shown. The three horizontal lines represent the thresholds for 70 °C, 72 °C and 74 °C.

Table 2 Correlation of the normalized blue peak intensity to temperature

Set-point temperature	Normalized blue peak intensity
70 °C	0.9014
72 °C	0.9965
74 °C	0.9632

is on the higher side of the TLC colour change range (*e.g.* 74 °C, Fig. 2c), the red peak is at the baseline (no increase in intensity).

We found that the variation in TLC signal is quite small (compared with the measurement uncertainties) until the temperature nears the edge of the operating range of the TLCs, whereupon the signal drops off rapidly. This means that the measurements near the desired temperature (at the centre of the operating range) provide relatively little information on the temperature *vs.* time behaviour and that most of this information is provided by the behaviour near the edges of the operating region. For example, when the temperature set-point is changed from 72 °C to 71 °C or 73 °C, the resulting change in the normalized blue intensity was found to be ~0.01. Since the uncertainty in measuring the normalized blue intensity is also 0.01 (as determined earlier), these points provide little information. Moving further away to 70 °C or 74 °C, a 1 °C change in temperature corresponds to a larger change of ~0.02 or more in the normalized blue intensity. Hence, the 0.01 uncertainty in the normalized blue intensity corresponds to a smaller uncertainty of ~0.5 °C (or less) in temperature. Temperature set-points located even further away fell outside the colour change range (bandwidth) of the TLCs and hence yielded no spectral change for analysis. As a result, three temperatures separated by steps of 2 °C (sufficiently large to ensure the variation in normalized blue intensity was greater than its uncertainty) and their normalized blue intensities were used as reference points (Table 2). The times at which the normalized blue intensity value crosses the reference values were noted (Fig. 2d), and translated into temperature crossing points. We obtain a temperature crossing point every time the normalized blue intensity *vs.* time plot crosses one of the reference values. For example, in Fig. 2d, every time the plot crosses 0.9014 and the red peak is present, we know the temperature has crossed 72 °C. The temperature and its time derivative must be continuous, and the PI/PD controller algorithm ensures that the temperature is slowly varying (changes of 1 °C s^{-1} or less) as it approaches the set-point value. Near any one extremum, we could readily fit a quadratic (*i.e.* a parabolic curve), but with successive extrema we need a higher order polynomial in order to take into account the nearby extrema. It is well-known that the higher the order of polynomial, the more likely one is to obtain spurious fluctuations. The lowest order polynomial that could fit the observed behaviour is a cubic. Hence, we fitted a cubic spline to these crossing points to obtain an estimate of the temperature *vs.* time trajectory during the transitions between temperature stages.

Results and discussion

For all the temperature transitions, we found that the temperature controller could be fine-tuned to find a good balance

between speed and overshoots/undershoots in the chamber temperature. Fig. 3a is representative of the results of such tuning: during the denaturation stage of the PCR, the chamber temperature just touches 96 °C (only one crossing point) and the undershoot is below 92 °C (two crossing points). This overshoot was deemed acceptable (too low to boil or cause extensive degradation of the enzyme) and the undershoot is thought to be of little consequence. However, without fine-tuning the temperature controller, overshoots or undershoots could be much larger and could impair operation. Fig. 3b is representative of an untuned transient and shows the temperature *vs.* time of the chamber during the annealing stage of the PCR, indicating that the chamber temperature overshot by 5 °C to about 55 °C (two crossing points at 58 °C) before settling back to 60 °C. In general, this is not acceptable, as such an overshoot is likely to cause non-specific amplification (this is application specific). The present TLC method can hence be used as feedback for fine-tuning the controller. The rapid (several second duration) transients found here are likely representative of the behaviours within many LOC implementations of PCR. In the present work, the slow settling to the steady-state (over ~60 s) results from the fact that a large unused region of the chip communicates thermally with the reaction chamber and comes to equilibrium gradually. Neither the rapid nor the slow transients were substantial enough (for this application) to be detrimental to the amplification of the β2 microglobulin (β2M) gene. However, in general, such rapid transients are likely to have a great impact on the reliability of more temperature sensitive PCRs.[1,2]

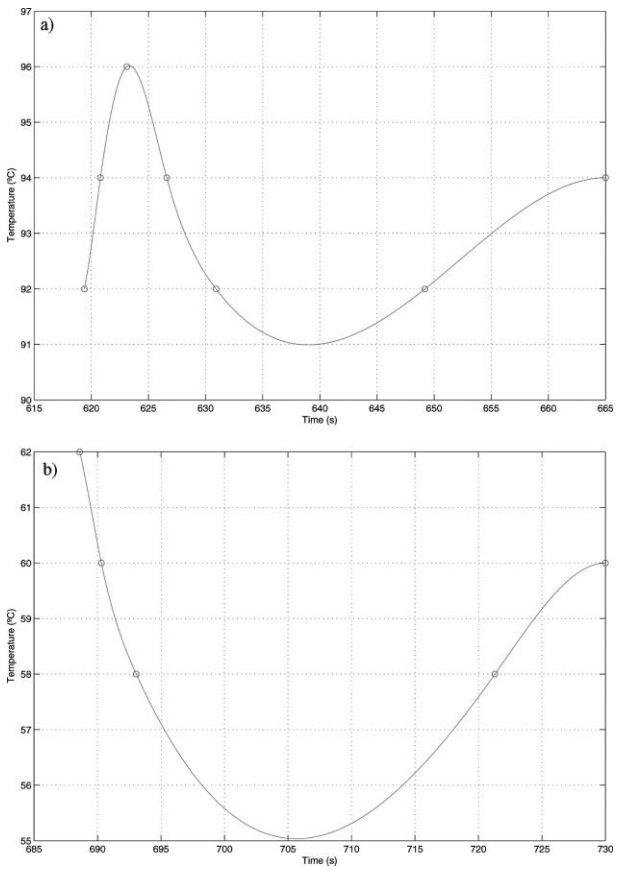

Fig. 3 Chamber temperature estimates and the dynamic trends as captured by the optical spectra of the TLCs during the transition (a) from the 72 °C stage to the 94 °C stage and (b) from the 94 °C stage to the 60 °C stage. For PCR, accurate temperature control is the most critical for these two transitions. The temperature controller had been fine-tuned in (a), but not in (b).

Concluding remarks

The present design is a suitable test-case for our dynamic TLC-based method: the volumes are relatively large (to ensure that sufficient template DNA is available when using clinical samples) and the bulk material is low-conductivity glass, resulting in long equilibration times and large temperature differences. These temperature differences may be small for high conductivity materials, such as silicon, but tens of degrees are common in glass-based applications.[6] Even larger temperature differences would be expected for polymer-based devices (lower thermal conductivity than glass). It is common that a sensed value of temperature at one point is used to determine the heat that should be applied at a second, often different point. As a result, we feel that the results found here are likely to be representative of the transients on many miniaturised PCR systems, particularly glass-based ones like the microchip studied here. It has been noted[6] that for temperature accuracy, one requires tight-bandwidth TLCs, however, such TLCs do not provide any information on the temperature beyond their bandwidth. There is clearly a significant benefit to estimating a temperature *vs.* time trajectory in a method that allows the use of tight bandwidth TLCs while monitoring for large transients (beyond the bandwidth) that may impair reliability. To improve the accuracy of the method, more data points might be obtained with lower uncertainties and analysed with a non-linear least squares method. Given the sensitivity of the PCR process to such transients, dynamic characterisation methods are needed in order to realize clinical-grade LOC PCRs. The methodology presented here may be automated to facilitate the fine-tuning of temperature controllers to reduce overshoots/undershoots.

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