Development of Fluorescent Thermometry Methods for Microfluidic Systems

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Abstract: The feasibility of employing laser-induced fluorescence (LIF) techniques to study thermal transport at the microscale is investigated. Two implementations of fluorescent thermometry are considered: a two-dye system and a single-dye, two-color system. In the two-dye implementation, a highly temperature-sensitive fluorescent dye (Rhodamine B) and a second dye (Sulforhodamine-101) with a different (weaker) temperature sensitivity are used in tandem to determine fluid temperature with high accuracy and low noise utilizing a pulsed Nd:YAG laser as an illumination source. While the fluorescence intensity of the temperature-sensitive dye is proportional to temperature, it is also biased by variations in the illuminating intensity. Therefore, the second dye with a different temperature sensitivity is required to compensate for such biases. Calibration of this two-dye LIF implementation reveals a temperature sensitivity of –1.5%/K and –2.7%/K when dissolved in ethanol and water, respectively, with volumetric illumination from a Nd:YAG laser. The feasibility of this methodology for conducting temperature measurements is explored by assessing the steady-state, linear temperature profile generated across a microfluidic channel array with two large hot and cold reservoirs. These measurements reveal that the mean steady-state temperatures in the microchannels are within ±0.4°C of the predicted temperatures with experimental uncertainties (based on standard deviation) of ±0.48–0.59°C (relative uncertainties of 1.4–2.0%). The second implementation (single dye, two color) allows one to account for variations in the illumination intensity by leveraging the temperature dependence of the emission of a single dye at different bands of wavelength. Preliminary assessment of this single-dye implementation with Sulforhodamine-101 reveals higher experimental uncertainties of ±0.78–0.93°C.

1. Introduction

The recent development of microfluidic systems like µ-TAS and µ-heat exchangers has led to a growing demand for the development of precision microscale measurement techniques to monitor both momentum and thermal transport. In such devices, the accurate assessment of temperature will not only aid in the optimization of their design, but will also maximize their efficiency, reliability, and productivity during operation (Kandlikar, 2003). For instance, precision control of fluid temperature by accounting for local temperature gradients could maximize the productivity of a microfluidic system associated with chemical operations such as mixing, reactions and separations. Such control becomes crucial when electrokinetic pumping is used for driving flow through these devices as the current flowing through the buffer solution can result in significant internal heat generation, a phenomenon known as Joule heating. Since most microfluidic-based MEMS devices utilize very high heat and mass transfer rates, a thorough understanding of their thermal transport characteristics is paramount for optimizing their design for increased performance and reliability.

While several methods exist for measurement of fluid temperature at the macroscale, the direct applications of these methods to the microscale may not be possible. In particular, accurate determination of instantaneous fluid temperatures and temperature gradients is extremely challenging at the microscale because dissipation of such gradients occurs over very short time scales due to high heat-transfer rates. Therefore, while several temperature measurement techniques exist for measurement of temperature at the macroscale where the limiting factors of thermal transport are less restrictive, methods with similar accuracy at the microscale are only now
being pursued. Most temperature measurements in microfluidic systems were limited, until recently, to measurements of bulk fluid temperature at the inlet and outlet of microfluidic sections or measurements of the substrate’s temperature itself. With regard to local temperature measurements, the use of high-precision thermocouple probes to measure fluid and/or substrate temperature is generally restrictive. In addition to being intrusive, these probes can suffer from poor spatial and temporal resolution since most probes have a characteristic size of a few microns. Alternatively, microfluidic devices can also be fabricated with integrated resistance temperature detectors (RTDs) embedded in the substrate with spatial extents on the order of a few microns (Wu et al., 1999). However, the fabrication procedure is often quite complex and RTDs suffer from poor spatial resolution which limits their ability to resolve local thermal gradients. Further, while these sensors are convenient for monitoring surface temperature, they do not provide a direct measure of the local fluid temperature.

One of the most accurate temperature-measurement methods at the macroscale is laser-induced-fluorescence (LIF). In this technique, a temperature-sensitive fluorescent dye (typically Rhodamine B) is dissolved within the fluid of interest and the dye is excited to fluoresce with an illumination source (Sakakibara et al., 1997). The local intensity of the fluoresced light is proportional to the local temperature of the fluid but with a limited accuracy of roughly ±2.0K, primarily due to variations in the intensity of the illumination source. To overcome such issues, Sakakibara and Adrian (1999) introduced a second dye into the solution whose fluorescence intensity was temperature-insensitive, yielding a local measure of incident intensity variations within their continuous illumination source. The fluoresced light from each dye excited with an Argon laser was imaged by separate cameras and the ratio of these intensities led to an estimate of temperature with a much-improved accuracy of ±0.2K (Sakakibara and Adrian, 2004; Funatani et al., 2004).

There are two main impediments associated with successfully implementing LIF methods at the microscale. First, most microfluidic systems, like lab-on-a-chip devices, generally only allow optical access from the top of the device (along the z direction if the channel network lies in the x−y plane). Therefore, one is forced to illuminate the test section from the z-direction which will inherently illuminate the entire depth dimension of the domain of interest. As has been observed extensively in microscopic particle image velocimetry (PIV) applications, volume illumination significantly increases the out-of-focus noise levels. Ross et al. (2001) attempted LIF temperature measurements via volume illumination with a continuous light source and a single temperature-sensitive dye in a microfluidic device and their system yielded a measurement accuracy of ±1.5K. However, it is not clear whether this limited accuracy was associated with the adverse effects of volume illumination, variations in illumination intensity, or both. Alternatively, one can employ a continuous micro-lasersheet (roughly 10 µm thickness) as an illumination source. Yoon and Kim (2006) designed a special microchannel with optical access from both the side and above and employed such a device for illumination of a much thinner region in the depth dimension for measurements of concentration fields by LIF. This methodology reduced the out-of-plane noise significantly, although it cannot be easily applied to complex channel networks. Further, temperature was not inferred from these LIF measurements, so it is not clear whether any improvements might be garnered with such an illumination methodology.

The second difficulty encountered in measurement of temperature at the microscale involves the thermal-transport time scales. Given that the time scale of heat dissipation at the microscale is quite short, coupled with the fact that the intensity of the incident light must be high enough to yield measurable fluorescence from the dyes, continuous illumination via an Argon laser (the standard for LIF at the macroscale) nor the aforementioned micro-lasersheet employed by Yoon and Kim (2006) are likely not appropriate. However, pulsed lasers, like the Nd:YAG laser, can provide sufficient illumination intensity over time scales much shorter than those of thermal transport at the microscale, meaning instantaneous measurements of temperature may be achievable. To this end,
LIF techniques are being developed to allow accurate measurements of instantaneous fluid temperature at the microscale. In particular, two implementations of fluorescent thermometry are considered: a two-dye, two-color system and a single-dye, two-color system. Although the two-dye methodology has been previously adapted to the microscale (Kim et al., 2003), these experiments were performed using a continuous Argon laser as an illumination source and a combination of Rhodamine B and Rhodamine 110 dyes. These parameters are identical to the experimental protocols commonly used at the macroscale where the thermal time scales are quite long. However, as outlined above, given the short time scales involved in thermal transport at the microscale, use of a continuous illumination source can severely limit the accuracy of temperature measurements at the microscale. Therefore, as a part of the current study we assess the feasibility of applying the two-dye LIF methodology at the microscale using a pulsed Nd:YAG laser emitting in the green as an illumination source. As with previous micro- and macro-scale studies, Rhodamine B is used as the temperature-sensitive dye since its absorption spectrum overlaps the emission wavelength of the Nd:YAG. In contrast, Rhodamine 110 cannot be used as the temperature-insensitive dye since its absorption spectrum occurs predominantly below the emission wavelength of the Nd:YAG. An alternative fluorescent dye is proposed to account for variations in incident illumination intensity and its spectral and temperature-dependent characteristics are documented. Additionally, the implementation of a single-dye, two-color system is examined wherein variations in the illumination intensity are compensated by utilizing a dye whose emission intensity exhibits temperature dependence as a function of wavelength. As such, by accounting for variations in illumination intensity, this study provides a measure of how detrimental volume illumination effects can be in microscale implementations of fluorescent thermometry.

2. Principles of LIF

When a photon of given energy is absorbed by a fluorophore, the energy state of the molecule transitions from the ground state to higher electronic states. Energy is subsequently dissipated at these excited states until the molecule reaches the lowest level of the first excited singlet state. Thereafter, the molecule at the first singlet state can return to the ground state in multiple ways. One such path of return is via fluorescence, defined as a radioactive decay process wherein no further energy is dissipated by collisions and the return of the excited molecule to the ground state occurs purely by the emission of energy. In such a scenario, a portion of the remaining energy is lost by the molecule before the emission and consequently, the emitted energy is of longer wavelength than the energy it initially absorbed. This shift in the wavelength of the fluoresced light relative to the absorption wavelength is referred to as the Stokes shift.

For a dye of concentration \( C \) (kg m\(^{-3}\)) illuminated with an incident light flux of intensity \( I_o \) (W m\(^{-3}\)), the fluorescence energy emitted per unit volume, \( I \) (W m\(^{-3}\)), is

\[
I = I_o C \phi \varepsilon, \tag{1}
\]

where \( \varepsilon \) is the absorption coefficient of the dye and \( \phi \) is its quantum efficiency. For most organic dyes, variations in \( I \) with temperature are predominantly attributable to the temperature dependence of the quantum efficiency. Therefore, in principle, if one can maintain \( I_o \) and \( C \) constant, the temperature of a fluorescent dye solution can be measured through variations of fluorescence intensity associated with the temperature dependence of \( \phi \). However, while maintaining a constant concentration is certainly achievable, it is impossible to maintain a constant incident light flux. Therefore, the fluorescence intensity not only embodies variations in solution temperature but also variations in the illumination intensity. Hence, the accuracy of fluid-temperature measurement with a single fluorescent dye is inherently limited by the homogeneity and stability of the illumination technique employed.
In order to alleviate accuracy limitations associated with variations in the illumination intensity, a second fluorescent dye, whose quantum efficiency has little, or no, temperature dependence is added to the solution and the fluorescence intensity of the two dyes is imaged simultaneously. Therefore, while the intensity of the fluoresced light from the temperature-dependent dye embodies both variations in temperature and illuminating intensity, any variations in the fluorescence intensity of the temperature-insensitive dye are directly attributable to variations in the illuminating intensity. If the emission from the two dyes, labeled A (temperature sensitive) and B (temperature insensitive), are separated perfectly into cameras α and β, the ratio of the fluorescence intensities recorded by both cameras is expressed as

\[
\frac{V^\alpha}{V^\beta} = \frac{I_A}{I_B} = \frac{C_A \phi_A \epsilon_A}{C_B \phi_B \epsilon_B},
\]

where \(V^\alpha\) and \(V^\beta\) are voltage outputs from the CCD’s of cameras α and β, respectively. This ratio is independent of the incident light flux \(I\) but depends on temperature via \(\phi_A/\phi_B\). In practice, however, it is impossible to completely separate \(I_A\) and \(I_B\), both because the emission spectra of most organic dyes are rather broad, meaning some overlap of their emissions is to be expected, and because spectral filters are inherently imperfect (filtering efficiencies of 90-95% are typical). Sakakibara and Adrian (1999) considered the case of imperfect separation of emission of two fluorescent dyes, wherein some fraction of the fluorescent intensity of the emission from dyes A and B are imaged by cameras β and α, respectively, and derived the expression

\[
\frac{V^\alpha}{V^\beta} = \frac{\phi_A \epsilon_A}{\phi_B \epsilon_B} \frac{C_A}{C_B} \frac{I_{\alpha}}{I_{\beta}} + \frac{\phi_A \epsilon_A}{\phi_B \epsilon_B} (1 - \frac{\phi_A \epsilon_A}{\phi_B \epsilon_B}) \frac{V^\alpha_{C=0}}{V^\beta_{C=0}},
\]

that accounts for this effect. Here, \(C_A^', \phi_A^', \alpha^C_{C=0}\), and \(V^\beta_{C=0}\) are the measured parameters for fluorescent dye A with \(C_B = 0\) and \(C_A^', \phi_A^', \alpha^C_{C=0}\), and \(V^\beta_{C=0}\) are the measured parameters for fluorescent dye B with \(C_A = 0\). In this context, \(V^\alpha_{C=0}\), \(V^\alpha_{C=0}\), \(V^\beta_{C=0}\) and \(V^\beta_{C=0}\) are constants for a given optical configuration. Therefore, in this case, the intensity ratio \(I_A/I_B\) is only a function of the concentration of the two dyes, \(C_A\) and \(C_B\), and the temperature, \(T\).

3. Experimental Setup

Figure 1 presents a detailed schematic of a typical experimental setup used for imaging the emission of the two dyes in a microfluidic device of interest. This setup is essentially identical to that commonly utilized in microscopic PIV save for an additional camera and additional filters. Light from an Nd:YAG laser (\(\lambda = 532\) nm, 12mJ, Continuum) is navigated through a microscope (Olympus BX60) to illuminate the test section. The fluorescence emission from the dyes passes through a 10× objective lens (Olympus UPlan Fl, N.A. : 0.30, W.D. : 10.0 mm, D.F. : 14.72 μm) followed by a long-pass filter (\(\lambda > 550\) nm, Chroma Filter) that removes all remaining incident light. The light fluoresced by each dye is then separated using a dichroic filter cube (\(\lambda = 600\) nm, Chroma Filter) which reflects wavelengths of \(\lambda < 600\) nm toward camera α and transmits larger wavelengths toward camera β. A short-pass filter (550 nm < \(\lambda < 580\) nm, Melles Griot) just upstream of camera α provides an additional filtering step before the fluoresced light is imaged. Similarly, an additional long-pass filter (\(\lambda > 665\) nm, Melles Griot) is positioned just upstream of camera β to further purify this fluoresced light prior to imaging. The cameras utilized in this effort are identical 12-bit, cooled CCD cameras with an effective chip area of 1396×1040 pixels (Photometric, CoolSnap HQ2) and are attached to the microscope using a dual-camera attachment (U-DPTS, Olympus) which maintains the same focal plane in both cameras. Precise synchronization between the firing of the laser pulses and the acquisition of images by both cameras is achieved with a digital delay generator (Model 500 Pulse Generator, BNC).
4. Fluorescent dye selection

There are several important factors that must be considered in the selection of appropriate dyes for a two-dye LIF system. First, the combination of a temperature insensitive dye and a strong temperature dependent dye is preferred since it will maximize $d(\phi_A/\phi_B)/dT$ (Sakakibara and Adrian, 1999, 2004). In addition, since the dyes are excited by a single illumination source, they should have similar absorption characteristics but quite different emission spectra. The latter requirement is crucial for efficient optical separation before imaging. Finally, the dyes must be efficiently dissolved in the solution and well-mixed at the molecular scale for accurate measurements of the intensity ratio at the microscale.

As part of the present effort, Rhodamine B (RhB) is chosen as the temperature-sensitive dye for the two-dye system. This dye is soluble in water as well as organic solvents such as ethanol and displays a rather strong temperature sensitivity ($-2.3\%/K$ and $-1.45\%/K$ when dissolved in water and ethanol, respectively). In addition, when dissolved in ethanol, RhB absorbs light at $\lambda_{abs} = 535$ nm and fluoresces at a wavelength of $\lambda_{em} = 565$ nm. These characteristics are quite advantageous in the present effort because the dye can be easily excited by an Nd:YAG laser emitting in the green but fluoresces at a higher wavelength, facilitating efficient separation of the emission wavelength from the illuminating wavelength. Sulforhodamine-101 (SR101) is selected as the second dye because of the large Stokes shift that it exhibits (Copetta and Rogers, 1998). In addition, its absorption spectrum is suitable for excitation using a Nd:YAG laser in the green ($\lambda_{abs} = 558$ nm) and its emission spectrum ($\lambda_{em} = 610$ nm) can be separated from both the illuminating wavelength and the emission wavelength of Rhodamine B when dissolved in ethanol. Finally, the fluorescence intensity of SR101 increases slightly with increasing temperature (Copetta and Rogers, 1998), in stark contrast to the temperature dependence exhibited by RhB. As such, the temperature sensitivity of the RhB/SR101 combination is actually higher than that achievable with RhB and a dye with no sensitivity to temperature.

Figure 2 presents the emission spectra of both dyes at two different temperatures, 20°C and 40°C, respectively, when excited at 532 nm. The shaded regions illustrate the wavelengths of light transmitted by the low-pass ($550 \text{ nm} < \lambda < 580 \text{ nm}$) and the high-pass ($\lambda > 665 \text{ nm}$) filters prior to imaging by cameras $\alpha$ and $\beta$, respectively. Both dyes have broad emission spectra with overlapping...
wavelengths in the range 570 nm < \lambda < 660 nm, with their emission peaks separated by 40 nm. Figure 2 illustrates that the emission intensity of RhB decreases over all wavelengths when the temperature of the dye is raised from 20°C to 40°C. On the other hand, the emission intensity of SR101 displays a temperature dependence that varies with wavelength. This dependence is strongest at lower wavelengths (\lambda < 610 nm) while little temperature dependence is noted at the higher wavelengths (\lambda > 610 nm) where the emission of SR101 is imaged in the present effort. It should also be noted that the current filtering arrangement (figure 1) nearly eliminates the emission of RhB from camera \beta (the imaging device for the SR101 emission), while camera \alpha (the imaging device for the RhB emission) captures a significant fraction of the SR101 emission. Therefore, equation (3) can be simplified to

$$\frac{I_A}{I_B} = \frac{V^\alpha - \varphi(T)V^\beta}{V^\beta}$$

(4)

where \varphi(T) = V^\alpha_{CA=0}(T)/ V^\beta_{CA=0}(T), represents the ratio of the emission from SR101 imaged by cameras \alpha and \beta, respectively, when C_{RhB} = 0. The current arrangement also facilitates the implementation of a single-dye two-color system with the emission from SR101 at the lower wavelengths and the higher wavelengths (acquired by cameras \alpha and \beta, respectively) serving as the temperature-sensitive and -insensitive emissions, respectively. Such an implementation of the two-color system is identical to the two-dye implementation described above save the fact that the intensity-ratio of the temperature-dependent and -independent portions of the emission from a single dye at the higher and lower wavelengths, respectively, is used to determine the temperature of the fluid.

Figure 3 shows the normalized emission intensities of RhB and SR101 acquired simultaneously using cameras \alpha and \beta, respectively, when the dye mixture is illuminated using successive laser pulses. Additionally, for each illumination pulse, the ratio of the emissions from RhB and SR101 are computed and presented in figure 3. Measurements of the emissions from both dyes are acquired in the interior of a PDMS microchannel of width 1 mm through which the dye mixture is flowed by means of a syringe-pump. Care is taken to ensure that the temperature of the dye mixture is kept constant at 20°C using a circulating water bath. By performing the measurements
in such a manner, it is ensured that the emissions from both dyes are not tainted by photobleaching effects and are independent of any variations in the fluid temperature. Therefore, any variations in the emissions from both dyes are directly attributable to fluctuations in the illumination intensity of the incoming laser pulses. Figure 3 illustrates that the emissions from RhB and SR101 vary by roughly ±6% about the mean intensity between successive laser pulses. In contrast, the intensity ratio of the simultaneous emission from both dyes exhibits relatively decreased scatter about the mean and reveals a variation of roughly ±1.5% about the mean value. Therefore, figure 3 reveals the efficacy of the two-color LIF technique in compensating for the fluctuations in the incoming illumination intensity from one laser pulse to another.

5. Results

5.1 Calibration

Figure 4 presents a schematic of the device used to calibrate the temperature dependence of the two dyes. A circular reservoir of diameter 20.0 mm and depth of 2.0 mm is filled with the dye solution and placed on an aluminum stage connected to a temperature-controlled water bath (TC-501, Brookfield). The side walls of the reservoir are insulated using silicon rubber and the top is covered by a 2.0-mm thick glass slide. The temperature of the dye solution inside the reservoir is monitored using a thermocouple (Type T, Quick-response PFA-Insulated Probes, Cole Palmer) and provides an independent measure of the fluid temperature inside the reservoir. At a known temperature, when illuminated using a Nd:YAG laser pulsed at 1 Hz with 12 mJ of energy per pulse, the emission from the dye solution is imaged by the camera and an ensemble of ten successive images of the fluorescence emission is acquired. An average over the ensemble and a spatial average over a region of 30 × 30 pixels is performed at each temperature to minimize variations in the intensity of the recorded fluorescence due to noise (attributable to the variations in the illuminating light as well as noise from the CCD array). This procedure is repeated until image ensembles are acquired at several known temperatures spanning the range 10–60°C. A single, average intensity value associated with each temperature is then obtained using the ensemble of the fluorescence emission acquired at that temperature.
Figure 4. Schematic of the device used to calibrate the temperature dependence of the dyes.

Figure 5. (a) Variation of $\phi$, the ratio of the emission from SR101 imaged by cameras $\alpha$ and $\beta$, respectively, as a function of temperature. (b) Temperature dependence of the fluorescence emission of Sulforhodamine-101 and Rhodamine B. The measured intensities are normalized by the intensity at 20°C ($I_{20}$).

As illustrated in equation (4), in order to obtain the variation of the intensity ratio, $I_A/I_B$, as a function of temperature, one must first obtain $\phi(T)$ (the ratio of the emission from SR101 imaged by cameras $\alpha$ and $\beta$, respectively, when $C_{RhB} = 0$). Figure 5(a) presents the variation of $\phi$ as a function of temperature, obtained by imaging the emission from SR101 alone (concentration of 0.01 mg/l in ethanol) using both cameras $\alpha$ and $\beta$. The variation of $\phi$ with temperature is linear and increasing at the rate of approximately 0.27%/K. Ideally, if the emission from SR101 over the range of wavelengths captured by cameras $\alpha$ and $\beta$ showed nearly identical temperature dependence, $\phi$ would be roughly constant over the entire range of temperature. The steady increase in the value of $\phi$ with increase in temperature is consistent with the trends of the emission spectra of SR101 observed in figure 2 which illustrate that the emission from SR101 at the lower wavelengths (acquired by camera $\alpha$) exhibits a stronger temperature dependence compared to the SR101 emission at the higher wavelengths (acquired by camera $\beta$).

Figure 5(b) presents the calibration of the fluorescence emission of RhB (concentration of 0.005 mg/l in ethanol) and SR101 (concentration of 0.01 mg/l in ethanol) as a function of temperature. The measured intensity values are normalized by the corresponding intensity values at 20°C. As expected, RhB shows a strong temperature dependence, with the fluorescence emission varying at the rate of approximately $-1.45$%/K. On the other hand, the calibration of SR101 shows a slight increase in the emission intensity with temperature of approximately $+0.14$%/K–consistent with its rather weak temperature dependence at higher wavelengths (figure 2). These normalized
intensity values, along with \( \phi \) in figure 5(a), are used to compute the intensity ratio, \( I_{\text{RHB}}/I_{\text{SR101}} \), at each temperature step using equation (4) which yields a calibration curve for the intensity ratio of the dye combination as a function of temperature. As illustrated in figure 5, the temperature sensitivity of the RHB-SR101 dye combination is approximately \(-1.50\%/K\)–slightly higher than the sensitivity of RHB alone. For the single-dye implementation which utilizes the temperature dependence of the SR101 emission at different wavelengths, the variation of \( \phi \) as a function of temperature [figure 5(a)], represents the calibration of the intensity-ratio of the temperature-dependent and -independent portions of the emission from SR101 with temperature and reveals a temperature sensitivity of roughly 1%/K.

Finally, it must be noted that prolonged exposure of fluorescent molecules to high intensity incident light can eventually lead to their degradation, a phenomenon known as photobleaching. Photobleaching effects are irreversible and can severely compromise the accuracy of quantitative fluorescence. In cases where such effects cannot be avoided, one can attempt to compensate for such losses by performing an \textit{a priori} photobleaching calibration that embodies these effects. Figure 6 presents the intensity of RHB and SR101 (concentration of 0.005 mg/l and 0.01 mg/l in ethanol, respectively) as a function of the number of successive laser pulses used to illuminate the dyes. The temperature of the dyes is kept constant at 20°C using a circulating water bath. Therefore, any variations in the emission intensity of the dyes are directly attributable to variations in the incoming illumination and photobleaching effects. In figure 6, the emissions from SR101 and RHB exhibit fluctuations on the order of approximately 5% about the mean intensity between successive laser pulses, consistent with the variability in the illumination intensity presented in figure 3. However, while the mean emission intensity of SR101 remains relatively constant for over eighty laser pulses, the mean emission from RHB decreases steadily after sixty laser pulses, indicating the onset of photobleaching effects in RHB. These results therefore suggest that photobleaching effects can be minimized in the present system by limiting the exposure of the same volume of the dye mixture to less than sixty laser pulses.

5.2 Temperature Measurements

The two-color LIF technique described above is implemented in the measurement of a one-dimensional, steady-state temperature gradient in a microfluidic device with a series of parallel
Figure 7. Schematic of the device used to obtain a one-dimensional, steady-state temperature gradient.

microfluidic channels [similar to the device employed by Mao et al. (2002)]. An array of seven parallel microchannels (width = 25 µm, depth = 100 µm; made of PDMS) spaced 175 µm apart are fabricated between two large reservoirs (width = 3.2 mm, depth = 3.2 mm) spaced 2 mm apart, that act as a heat source and heat sink, respectively (figure 7). Continuous circulation of heated and cooled water through the reservoirs ensures that they remain at constant temperatures. An independent measure of the temperature of the heated and cooled walls on either side of the channel array is obtained by inserting two thermocouples into the device through the hot and the cold reservoirs, respectively. As found by Mao et al. (2002), such an arrangement generates a linear temperature gradient across the channel array which is filled with the dye mixture. When illuminated with the Nd:YAG laser, a measure of the temperature of the dye mixture in each channel is obtained by imaging the fluorescence emission from the dyes.

Figures 8(a) and (b) present the variation of the steady-state temperature distribution across the microchannel array imposed by the hot and cold reservoirs obtained using the two-dye and the single-dye two-color systems, respectively. In order to conduct the measurements, the optical configuration illustrated in figure 1 is utilized for acquiring the emission from the dye mixture within the microchannels. For the two-dye implementation, emissions from RhB and SR101 are acquired simultaneously as image pairs using cameras α and β, respectively. Upon acquisition of each image pair, the emission imaged by both cameras is separated into its RhB and SR101 counterparts using equation (4). Finally, the ratio of the emission intensities of RhB and SR101 are computed for the intensity values recorded at each pixel location of the image and these intensity ratios are converted to their corresponding temperature values using the calibration curves presented in figure 5. For the single-dye implementation [figure 8(b)], the emission from SR101 at the lower and higher wavelengths are acquired by cameras α and β, respectively. The ratio of the emission intensities of SR101 acquired by both cameras are computed and converted into their temperature values using the calibration presented in figure 5(a). In both cases, an ensemble of sixty image pairs is acquired upon the attainment of a steady-state temperature distribution across the hot and the cold reservoirs, yielding sixty statistically-independent measurements of the temperature distribution across the microfluidic device.
Figure 8. Variation of temperature across the microfluidic device shown in figure 7 as measured using (a) the two-dye and (b) the single-dye, two-color systems. The solid circles indicate the mean temperatures obtained via two-color LIF measurements while the solid line indicates the predicted linear temperature gradient across the channel array based on independent thermocouple measurements of the reservoir temperatures. The error bars indicate measurement uncertainties obtained by computing the standard deviation of the data sets (Table 1).

In figures 8(a) and (b), the filled circles illustrate the mean temperatures of the dye mixture in the microchannel array obtained by averaging over the sixty samples, while the predicted linear temperature variation across the device (illustrated using a solid line in figure 8) is obtained by fitting a line through the independently-measured wall temperatures of the hot and the cold reservoirs for each of the two experiments. A measure of the uncertainty in the measurements is obtained by computing the standard deviation of the data sets comprising the ensemble (Table 1) lists the mean and the standard deviation for the temperature measurements in each of the seven microchannels of the microfluidic device. Figure 8(a) illustrates that the mean temperature of the dye mixture in each of the seven microchannels of the microfluidic device measured using the two-dye system, as indicated by the solid circles, is in very good agreement with the predicted temperature distribution (span of error bars represent two standard deviations). As illustrated in table 1, the mean temperatures are within ±0.4°C of the predicted temperatures, while the standard deviations vary from ±0.48°C to ±0.59°C. On the other hand, for the measurement performed using the single-dye methodology [figure 8(b)] reasonable agreement is observed between the measured values of the mean temperature of SR101 in each of the seven channels and that predicted using the linear temperature profile. The standard deviations of these measurements are observed to vary from ±0.78 °C to ±0.93 °C – higher than that observed for the measurements conducted using the two-dye system.

An estimate of the relative uncertainty is attained by normalizing the standard deviations by the measured temperatures. This normalization indicates relative measurement uncertainties of 1.4–2% and 2–3.2% for the two-dye and the single-dye, two-color systems, respectively, with the relative uncertainty increasing with decreasing temperature. The higher degree of variability noted in the single-dye, two-color SR101 measurements is consistent with its weaker temperature sensitivity (≈1%/K) compared to the two-dye system (≈−1.5%/K). Finally, contrasting the absolute uncertainties of ~±0.5°C and ~±0.85°C for the two-dye and single-dye, two-color implementations, respectively, with the ±1.5°C reported by Ross et al. (2001) for a single-dye, single-color volume-illumination microscale implementation indicates that a sizable portion of their error is likely due to variations in illumination intensity rather than volume illumination. Nevertheless, volume illumination plays a non-trivial role in microscale fluorescent thermometry as the uncertainties reported herein are still 2–3 times larger than those reported for macroscale two-dye LIF (Sakakibara and Adrian, 2004).
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Table 1. Temperature distribution across the channel array.

6. Summary

The results presented herein demonstrate the capability of two-color fluorescent thermometry techniques for accurate measurement of fluid temperature at the microscale. Due to the short thermal transport time scales that exist at the microscale, an Nd:YAG pulsed laser is employed as the illumination source. In particular, the experimental uncertainties associated with temperature measurements using two different implementations of the two-color system (two-dye and the single-dye, two color) are documented so that the errors associated with the variability in the intensity of the illuminating light are minimized. For the two-dye methodology, Rhodamine B and Sulforhodamine-101, with ethanol as the solvent, are used for the temperature measurements and this combination of dyes yields a temperature sensitivity of approximately $-1.50%/K$. For the single-dye system, two-color implementation, the ratio of the emission from SR101 at the lower and the higher wavelengths yields a temperature sensitivity of roughly $1%/K$. The capability of these systems in measuring fluid temperature at the microscale is demonstrated by conducting preliminary measurements of a one-dimensional steady-state temperature gradient in a microfluidic device with a series of parallel microfluidic channels. These measurements reveal experimental uncertainties ranging from $\pm0.5–0.63^\circ C$ and $\pm0.78–0.93^\circ C$ for the two-dye and the single-dye, two color systems, respectively.

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References


