

Generalized Temperature Measurement Equations for Rhodamine B Dye Solution and Its Application to Microfluidics

Jayna J. Shah,* Michael Gaitan, and Jon Geist

Semiconductor Electronics Division, National Institute of Standards and Technology, 100 Bureau Drive, MS 8120, Gaithersburg, Maryland 20899

Temperature mapping based on fluorescent signal intensity ratios is a widely used noncontact approach for investigating temperature distributions in various systems. This noninvasive method is especially useful for applications, such as microfluidics, where accurate temperature measurements are difficult with conventional physical probes. However, the application of a calibration equation to relate fluorescence intensity ratio to temperature is not straightforward when the reference temperature in a given application is different than the one used to derive the calibration equation. In this report, we develop and validate generalized calibration equations that can be applied for any value of reference temperature. Our analysis shows that a simple linear correction for a 40 °C reference temperature produces errors in measured temperatures between -3 to 8 °C for three previously published sets of cubic calibration equations. On the other hand, corrections based on an exact solution of these equations restrict the errors to those inherent in the calibration equations. The methods described here are demonstrated for cubic calibration equations derived by three different groups, but the general method can be applied to other dyes and calibration equations.

The temperature-sensitive nature of molecular fluorescence has been employed in a variety of research and industrial applications to provide spatial and temporal measurements of temperature.^{1–5} This approach is particularly suitable for temperature sensing in small volumes (e.g., microfluidic channels), hostile environments (e.g., microwave-irradiated system), or high-voltage systems where macroscopic physical probes have limited utility. The temperature measurement is based on measuring fluorescence intensity ratios. A suitable dye can be selected for a specific application as the fluorescence intensity of many fluorescent dyes is temperature dependent.^{1,6} Aqueous solutions containing single fluorophores

as well as a mixture of two fluorescent dyes, each having different temperature dependency, have been used for measuring fluid temperature.^{7–9} For a single fluorophore system, the fluorescence intensity is typically measured at a known reference temperature, which is later used to normalize the intensity measured after heating the medium to an unknown temperature. The temperature is extracted by substituting the normalized intensity into a calibration curve.

For lab-on-a-chip applications,^{10–13} the use of dilute solutions of a temperature-sensitive fluorescent dye, particularly Rhodamine B (RhB), has become very popular for optical measurement of temperature distributions. RhB is a fluorescent, water-soluble compound with an absorption peak at 554 nm, an emission peak at 576 nm, and a temperature dependent fluorescence quantum yield.¹⁴ The chemical properties of RhB have been studied extensively; its high temperature sensitivity, negligible pressure sensitivity, and nominal pH-independent absorption above pH 6 have been widely employed in a variety of fluidic applications.^{11–13,15} For example, Ross et al. have demonstrated the use of a RhB dye solution for measuring temperatures in microfluidic channels with high spatial and temporal resolution.¹⁰ Erickson et al. have employed a RhB solution to examine in-channel temperature and flow profiles at a T-shaped microchannel intersection during electrokinetic pumping.¹⁶ Seger-Sauli et al. have used a RhB dye solution in microchannels to investigate temperature induced risks for hyperthermic stress or cell damage.¹⁷ Fu et al. have utilized a RhB solution to characterize the temperature field resulting from resistive microheaters embedded in a poly(dimethylsiloxane) (PDMS) microchip.¹⁸ While RhB has been commonly used for temperature measurement in aqueous environment, Samy et al. have recently shown that absorbed RhB dye molecules in a PDMS

* To whom correspondence should be addressed. Jayna J. Shah, National Institute of Standards and Technology, Semiconductor Electronics Division, 100 Bureau Dr., MS 8120, Gaithersburg, MD 20899. Phone: 301-975-2305. E-mail: jayna.shah@nist.gov.

- (1) Lou, J. F.; Finegan, T. M.; Mohsen, P.; Hatton, T. A.; Laibinis, P. E. *Rev. Anal. Chem.* **1999**, *18*, 235–284.
- (2) Gallery, J.; Gouterman, M.; Callis, J.; Khalil, G.; McLachlan, B.; Bell, J. *Rev. Sci. Instrum.* **1994**, *65*, 712–720.
- (3) Sakakibara, J.; Hishida, K.; Maeda, M. *Exp. Fluids* **1993**, *16*, 82–96.
- (4) Ali, M. A.; Moghaddasi, J.; Ahmed, S. A. *Appl. Opt.* **1990**, *29*, 3945–3949.
- (5) Kubin, R. F.; Fletcher, A. N. *J. Lumin.* **1982**, *27*, 455–462.
- (6) Coppeta, J.; Rogers, C. *Exp. Fluids* **1998**, *25*, 1–15.

- (7) Sakakibara, J.; Adrian, R. J. *Exp. Fluids* **1999**, *26*, 7–15.
- (8) Finegan, T.; Laibinis, P. E.; Hatton, T. A. *AIChE J.* **2006**, *52*, 2727–2735.
- (9) Ebert, S.; Travis, K.; Lincoln, B.; Guck, J. *Opt. Express* **2007**, *15*, 15493–15499.
- (10) Ross, D.; Gaitan, M.; Locascio, L. E. *Anal. Chem.* **2001**, *73*, 4117–4123.
- (11) Shah, J. J.; Sundaresan, S. G.; Geist, J.; Reyes, D. R.; Booth, J. C.; Rao, M. V.; Gaitan, M. *J. Microeng. Microeng.* **2007**, *17*, 2224–2230.
- (12) Gui, L.; Ren, C. L. *Appl. Phys. Lett.* **2008**, *92*.
- (13) Low, P.; Kim, B.; Takama, N.; Bergaud, C. *Small* **2008**, *4*, 908–914.
- (14) Ferguson, J.; Mau, A. W. H. *Aust. J. Chem.* **1973**, *26*, 1617–1624.
- (15) Ross, D.; Locascio, L. E. *Anal. Chem.* **2002**, *74*, 2556–2564.
- (16) Erickson, D.; Sinton, D.; Li, D. Q. *Lab Chip* **2003**, *3*, 141–149.
- (17) Seger, U.; Panayiotou, M.; Schnydrig, S.; Jordan, M.; Renaud, P. *Electrophoresis* **2005**, *26*, 2239–2246.
- (18) Fu, R.; Xu, B.; Li, D. *Int. J. Therm. Sci.* **2006**, *45*, 841–847.

Table 1. Properties of Different RhB Solutions Used by Three Different Authors for Fluorescence-Based Temperature Measurements^a

	[RhB] (mmol/L)	[buffer] (mmol/L)	pH	A_0 (°C)	A_1 (°C)	A_2 (°C)	A_3 (°C)	T_0 (°C)	RT (°C)
Ross et al.	0.1	20	9.4	132	-250	220	-79	23	22
Fu et al.	0.05	25	8.5	149.15	-317.84	323.41	-131.84	22.88	23.5
Samy et al.	1.0,5.0	none	N/A	141.53	-250.25	228.02	-96.904	22.396	23

^a The coefficients (A_0 – A_3) of the third order polynomials fitted to the measured normalized fluorescence intensity versus temperature reported by those authors, the temperature T_0 given by their polynomials when the normalized fluorescence intensity ratio = 1.0, and the room temperature RT used for normalization. The calibration curves that are produced by the different polynomials for the different RhB chemistries used by these authors are plotted in Figure 1 for comparison.

thin film can be used for whole chip temperature measurement.¹⁹ However, this absorption of dye molecules depends on a number of factors including the pH, buffer ion concentration, and temperature, to name a few, and can lead to erroneous results and affect overall reliability and repeatability of the measurement if improperly treated. Glawdel et al. have addressed this issue by removing the unwanted fluorescence from absorbed dye molecules by photobleaching the area of interest prior to making temperature measurements.²⁰

Even though the temperature-sensitivity of fluorescent dyes has been utilized in a variety of applications, the calibration equations that are currently available for computing temperature relate the fluorescence intensity at an unknown temperature to the intensity at only one particular reference temperature. Such relations are not directly applicable to applications requiring a different reference or initial temperature. The purpose of the present correspondence is to generalize existing single-dye calibration equations, extending their use to fluorescence intensity data normalized to reference temperatures other than those for which the original calibration equations were derived. Two methods are described in detail: one is approximate while the other, based on solution of a cubic equation, is an accurate mathematical treatment that does not incur errors beyond those already inherent in the calibration equations. Finally, the uncertainties in the existing calibration equations as well as the potential for improved accuracy are discussed.

GENERALIZATION OF RHODAMINE B TEMPERATURE EQUATIONS

Let $S(T)$ represent the signal received from a fluorescence detection system observing a small volume of fluorescent species at temperature T and let

$$I_{RT}(T) = S(T)/S(RT) \quad (1)$$

represent the fluorescence intensity ratio normalized to the signal measured at nominal room temperature RT.

Ross et al. measured $S(T)$ for 0.1 mmol/L RhB in 20 mmol/L carbonate buffer from 15.9 to 90.4 °C. Fu et al. measured $S(T)$ for 0.05 mmol/L RhB in 25 mmol/L carbonate buffer solution from approximately 23.5 to 95 °C, and Samy et al. measured $S(T)$ of 30 and 60 μm thick PDMS thin films that had been immersed in 1 and 5 mmol/L RhB solution prepared in DI water, respectively. For convenience, this information is summarized in Table 1. Each author normalized the fluorescence intensity acquired from an

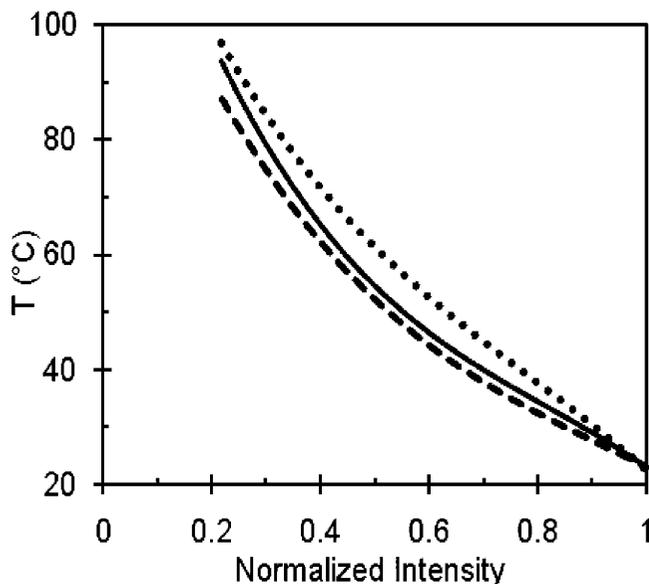


Figure 1. Comparison of the relative fluorescence-intensity versus temperature calibrations published by Ross et al.¹⁰ (---), Fu et al.¹⁸ (—), and Samy et al.^{10,18,19} (···) for different rhodamine B chemistries.

image captured at an elevated temperature to the intensity acquired from an image captured at nominal room temperature RT. This was followed by a least-squares adjustment of the constants in the equation

$$T = A_0 + A_1 I_{RT}(T) + A_2 I_{RT}^2(T) + A_3 I_{RT}^3(T) \quad (2)$$

to fit the measured $I_{RT}(T)$ for different values of T . Table 1 includes the values of A_0 to A_3 that were reported by Ross et al., Samy et al., and Fu et al., the temperatures T_0 given by eq 2 when $I_{RT}(T) = 1.0$, as well as the RT used by each of the authors for normalization purposes. These temperatures differ slightly from the normalized temperatures RT used by the different authors because their calibration equations were not constrained to produce RT when the intensity ratio was 1.0. The calibration curves from all three authors are compared in Figure 1. The general trend of all three curves is similar. The temperature dependence of the fluorescence of adsorbed dye molecules would not necessarily be expected to follow that measured for similar molecules in the bulk of the buffer.²¹ Hence, the difference between the curve of Samy et al. and the other

(19) Samy, R.; Glawdel, T.; Ren, C. L. *Anal. Chem.* **2008**, *80*, 369–375.

(20) Glawdel, T.; Almutairi, Z.; Wang, S.; Ren, C. *Lab Chip* **2009**, *9*, 171–174.

(21) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer Science + Business Media, LLC: New York, 2006.

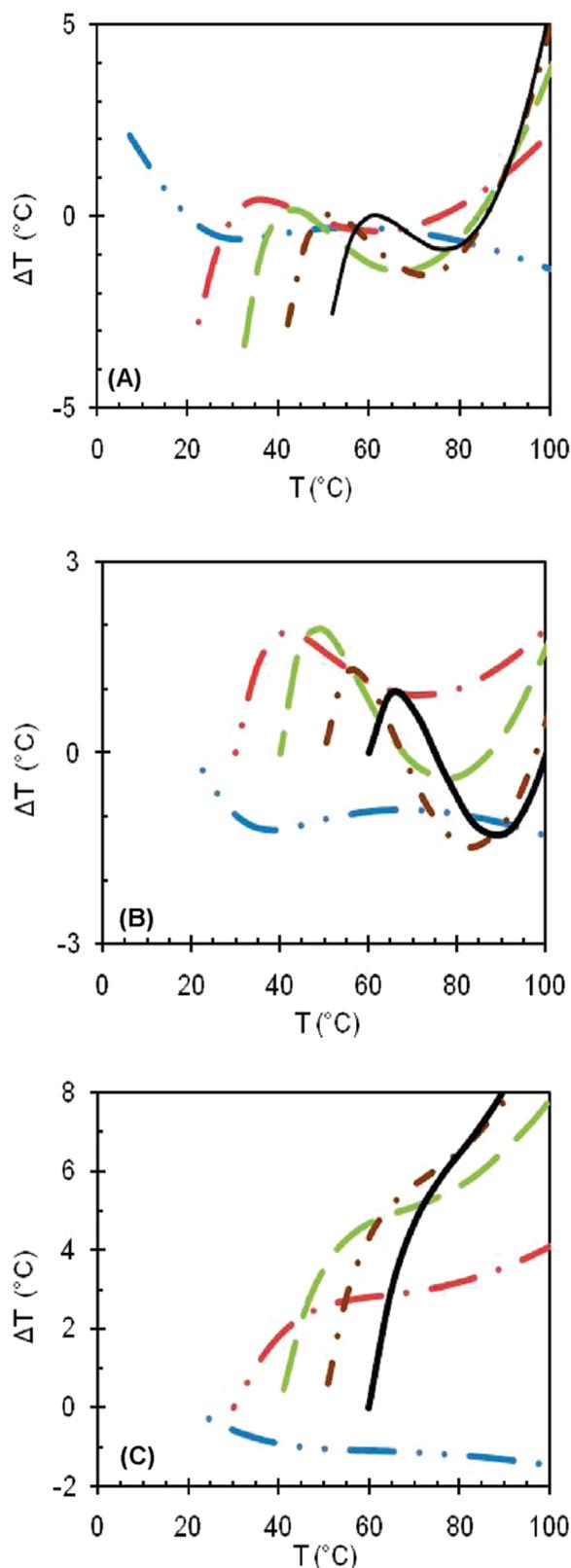


Figure 2. The excess temperature error (beyond that inherent in the relative fluorescence versus temperature calibration equations) produced by estimating temperature using I_{T_1} instead of I_{RT} in eq 2 and adding $T_1 - T_0$ to the final result. (A) The calibration equation published by Ross et al., (B) the calibration equation published by Samy et al., and (C) the calibration equation published by Fu et al. The coefficients used for constructing the plot can be found listed in Table 1. (— · · ·) $T_1 = 20$ °C, (— · ·) $T_1 = 30$ °C, (— — —) $T_1 = 40$ °C, (— · · · ·) $T_1 = 50$ °C, and (—) $T_1 = 60$ °C.

two authors can be explained by the different physical medium and local environment used in the measurements.

GENERALIZED CALIBRATION EQUATION

As mentioned previously, the fluorescence intensity measurement at a reference temperature in the vicinity of 23 °C for normalization purposes is not always practical. For instance, some applications require rapid temperature cycling of fluidic solutions where the cycling temperatures of interest are significantly different from 23 °C. In these situations, it is useful to calculate the fluorescence intensity ratio at a convenient reference temperature, T_1 , in the temperature range of interest as

$$I_{T_1} = I_{T_1}(T) = S(T)/S(T_1) \quad (3)$$

While it might appear plausible to use I_{T_1} in eq 2 instead of $I_{RT}(T)$ and to add $T_1 - T_0$ to the result to estimate the temperature, this process will add some excess error to the calculated temperatures as shown in Figure 2 for the calibration equations of Ross et al., Fu et al., and Samy et al., respectively. Notice that the excess error introduced by this procedure is zero when $T_0 = T_1$, but that this error can be larger than ± 1 °C in certain temperature regions for some values of T_1 for all three calibration equations. Also, the error introduced by this simple procedure is more serious for the calibration equation of Samy et al. than for the other two calibration equations.

With a different procedure, it is possible to eliminate all excess error except that inherent to the calibration equations themselves. For this case, we generalize the normalized fluorescence intensity by rewriting it as

$$I_{RT}(T) = \frac{S(T)}{S(RT)} = \frac{S(T)}{S(T_1)} \frac{S(T_1)}{S(RT)} = I_{T_1}(T) I_{RT}(T_1) \quad (4)$$

where T_1 is any convenient known reference temperature. Therefore, if $I_{T_1}(T)$ data have been measured where T_1 is not the reference temperature used in deriving the calibration equation, then $I_{RT}(T)$ can be calculated for use in eq 2 from eq 4, where $I_{RT}(T_1)$ can be obtained from the real solution²² of the cubic equation

$$0 = A_3 I_{RT}^3(T_1) + A_2 I_{RT}^2(T_1) + A_1 I_{RT}(T_1) + A_0 - T_1 \quad (5)$$

with the values of A_n as listed in Table 1 and

$$I_{RT}(T_1) = \frac{-A_2}{3A_3} + (R + \sqrt{D})^{1/3} + (R - \sqrt{D})^{1/3}$$

$$D = Q^3 + R^2$$

(22) Weisstein, E. W. Cubic Formula. From *MathWorld: A Wolfram Web Resource*; 2008, <http://mathworld.wolfram.com/CubicFormula.html>. Equations 1, 21, 22, 48–50, and 53 where Weisstein's $a_2 = A_2/A_3$ and $a_0 = (A_0 - T_1)/A_3$ in terms of the parameters introduced in this note. Last accessed November 3, 2008.

$$Q = \frac{3A_1A_3 - A_2^2}{9A_3^2}$$

and

$$R = \frac{9A_1A_2A_3 - 27(A_0 - T_1)A_3^2 - 2A_2^3}{54A_3^3} \quad (6)$$

When $I_{RT}(T)$ is calculated from eq 4 with measured $I_{T_1}(T)$ data and $I_{RT}(T_1)$ obtained from the solution to eq 5 that is given in eq 6, no error is introduced into the result beyond that already inherent in eq 2. A treatment similar to that described above can be applied to generalize calibration equations based on linear² and second order polynomial¹⁶ fits to normalized $I(T)$ data to a convenient reference temperature.

CONCLUSIONS

Two methods have been presented for generalizing temperature calibration equations for Rhodamine B dye solution, both of which are easy to apply. The accuracy of the method based on the exact solution to the cubic equation, however, depends merely on the accuracy of the calibration equation in use and does not introduce any error beyond that inherent in the calibration

equation itself. The residual standard deviation of the fit reported by Ross et al. was 0.8 °C with contributions from both the scatter in the measured data and the shape of the function used to fit the measured data. It should be possible to reduce this number by at least a factor of 10 with more accurate bath-temperature measurements, finer temperature resolution, more replications at each temperature, and careful control of solution chemistry. If more accurate calibration equations are developed in the future, use of the exact procedures to renormalize fluorescence intensity ratio data acquired at a temperature other than that used in determining the calibration equation will become even more important.

ACKNOWLEDGMENT

The authors gratefully acknowledge helpful conversations with Dr. David Ross (NIST). This research was performed while J.J.S. held a National Research Council (NRC) Research Associateship Award at the Electronics and Electrical Engineering Laboratory (EEL) of NIST. The authors are also thankful to the Office of Science and Technology of the National Institute of Justice for providing part of the support for this work.

Received for review July 23, 2009. Accepted August 18, 2009.

AC901644W