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基于荧光强度比值法可用于现场测量的低成本聚合物光纤温度传感器

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摘 要:基于荧光强度比值法,设计了一种使用两种荧光染料的光纤温度传感器.实验中,罗丹明 B 和罗丹明 110 分别为对温度敏感和对温度不敏感的荧光传感物质,利用聚合物光纤来传导激发光及接收荧光.由于两种染料的荧光谱峰相距 60 nm,因此容易将二者对应的荧光谱分开.通过确定能代表两种染料的最优荧光光谱范围,获得具有良好线性度的温度-荧光强度标定曲线.实验研究了不同浓度的荧光染料对标定曲线的影响,当染料浓度为 0.3 g/L 时,可获得 0.28℃的最小均方误差及 0.0128/℃的灵敏度.此外,该传感器还具备一定的抗光源扰动和抗荧光染料漂白的能力.

关键词:荧光强度比值;聚合物光纤;双荧光;温度传感器;荧光基团浓度
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Low-cost Robust Polymer Optical Fiber Temperature Sensor Based on FIR Method for in Situ Measurement

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Abstract: An optical fiber temperature sensor using two fluorescent dyes based on Fluorescence Intensity Ratiometric (FIR) method was proposed. In experiment, Rhodamine B and Rhodamine 110, which were temperature-sensitive and temperature-insensitive respectively, acted as the sensing materials. Polymer optical fibers were utilized to transmit excitation light and collect emitted fluorescence. The two dyes-fluorescence spectra could be separated conveniently since their respective emission peaks were 60 nm apart. The optimal spectral intervals of RH110 and RHB for FIR were identified. By calculating the fluorescence intensity ratio of the two dyes, the calibration curves of intensities ratios vs temperature were obtained with good linearity. The effects of different concentrations of fluorophore on the calibration curves were also studied. When the concentrations of dyes were 0.3 g/L, a minimum rms temperature error of 0.28℃ and a sensitivity of 0.0128/℃ were achieved. Moreover, the influences of illumination source-s fluctuations and dyes- photo-bleaching can be eliminated to some degree.

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0 Introduction

Fiber-optic temperature sensors have attracted much attention due to their advantages over the electronic counterparts, such as compact size, high safety, and immunity to Electromagnetic Interferences (EMI). The temperature sensors based on silica optical fibers can be implemented in a number of ways, e. g. fiber grating, macro-bend fiber loop, and F-P interferometry, etc.[1-4]. Compared with silica fibers, Plastic Optical Fibers (POFs) are more low-cost, flexible and easier to fabricate. As for the POFs temperature sensors, the precise measurement is achieved by detecting the shift of spectrum[5-7], where a complex system is usually applied. As we all know, the POF sensors are always established based on intensity modulation technique with simple configurations. Hence, in real application, an intensity-based POF temperature sensor with simple structure is expected to acquire precise measurement, and the fluorescence method is a promising approach to this goal.

Generally, there are three schemes that can be employed to measure the temperature using fluorescence method, which are the steady state fluorescence intensity method, the fluorescence lifetime method, and the Fluorescence Intensity Ratiometric (FIR) method. The steady fluorescence intensity method is the most direct way. However, the measurement results may be influenced by some artefacts due to the non-temperature related changes in the fluorescence intensity[8-9]. With the lifetime method, the high precision could be achieved, but a pulsed light source and fast response electronics are required and thus the setup would be costly[10]. The fluorescence intensity ratiometric method has been studied extensively due to its ability to mitigate or eliminate the external perturbation, e. g. the power fluctuation of the excitation light source, the variation of the transmission loss in optical pass, and the photo-bleaching effect of the dyes[11-15]. The FIR method, which is mainly applied in those fiber-optic sensors with rare earth materials doped in optical fibers, involves utilizing the fluorescence intensities from two closely spaced energy levels of an ion to monitor temperature[11,13-14]. When these levels are excited, their relative populations normally follow a Boltzmann distribution. Since the intensity from each level is proportional to its population, the fluorescence intensity ratio of the pair of levels is obtained, which can be used for temperature calibration. Meanwhile, in this scheme, two kinds of fluorophore, which are temperature-dependent and temperature-insensitive respectively, are utilized to calibrate temperature. Ideally, it can be assumed that the photo-bleaching effect has the same impact on the fluorescence bands for the two kinds of fluorophore[16]. As a result, the intensity ratio of the two corresponding fluorescence bands only depends on temperature. Hence, the FIR method is a technique that can provide a self-referenced measurement, which is beneficial for the accurate and reliable performance of the sensor system. As the two fluorophore used in Ref. [16]. tris (1, 10-phenanthroline) Ruthenium (II) (Ruphen) and 8-Aminopyrene-1, 3, 6-Trisulfonic acid (APTS), are temperature-sensitive and temperature-insensitive respectively, their fluorescence intensities ratio can also be obtained for temperature measurement, with the influences of photo-bleaching, pump power fluctuations and fiber bend removed. Similarly, another pair of fluorescent dyes, Ruphen and Rhodamine 123, is reported[17]. However, Ruphen is prone to oxidation hence it is usually encapsulated in silica gel. The silica gel would expand or contract as temperature varies, which results in the change of molecule distances of different fluorophore in it, and this would lead to unreliability during measurement. Moreover, the fabrication of mixing silica gel with fluorophore is complex. In addition, Ruphen is 2300 US$ per gram and APTS is 42400 US$ per gram, which are too costly.

In this paper, two fluorescence dyes, Rhodamine B (RHB) and Rhodamine 110 (RH110), which are temperature-dependent and temperature-insensitive, are encapsulated and immobilized on the tips of two glued polymer optical fibers to form a sensing head. With the FIR method, precise temperature measurement is realized.

1 Configuration and principle

As shown in Fig. 1, the sensor system consists of four parts-the illuminator, light guided fibers, sensing head and a compatible fiber-optic spectrometer (AverSpec-2048, Netherlands). The illumination wavelengths are obtained by a Tungsten Halogen lamp (AvaLight-HAL) followed by a band-pass optical filter which transmitted the wavelengths of 470～500 nm and blocked other wavelengths. The fluorescent dyes,

Rhodamaine B and Rhodamine 110, were dissolved in deionized water to form the sensing liquid. The light guided fibers used in our experiment are polymer optical fibers (POFs, Super Eska SK-40, Mitsubishi Rayon Co.). The core and cladding materials are Polymethyl-Methacrylate Resin (PMMA) and fluorinated polymer, respectively. The typical values for core and cladding diameters are 980 mm and 1000 mm, with NA of 0.5. As the transmission wavelengths of the POFs range from 400 nm to above 700 nm, including the wavelength of illumination light and fluorescence, the effect of transmission losses on fluorescence spectrum is negligible[18]. Two POFs with 1 m long, whose ends were polished and glued together by epoxy resin, were dipped into a stainless tube directly, in which the sensing liquid was encapsulated.

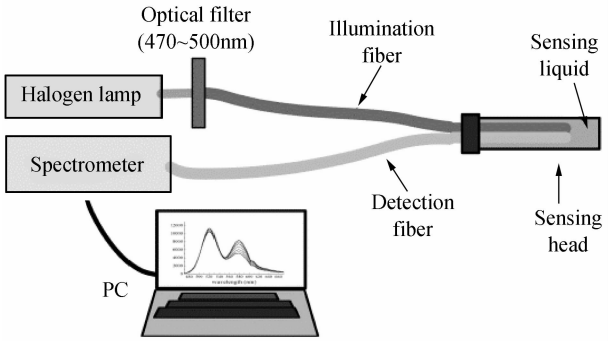


Fig. 1 The schematic structure of an optical fiber temperature sensor using the fluorescence signals from two fluorescent dyes

As illustrated in Fig. 1, the sensing head and fibers are fixed by epoxy glue, so it needs no further adjustment for coupling between the fibers and sensing head. One of the fibers acts as the excitation light fiber and the other serves as the detection fiber. When the temperature varies, the corresponding spectra are recorded by the spectrometer. When exposed to excitation light, each dye yields fluorescence. RHB is highly sensitive to the changes in the temperature in the range of 25-60℃, while RH110 has negligible sensitivity to temperature in this region. The fluorescence intensity is expressed as follows[19-20]

$$I = I_0 \alpha \phi \varepsilon L C$$

where, I is the emitted fluorescence intensity of the dyes, I_0 is the excitation light intensity, ϕ is the quantum efficiency of the fluorophore, ε is the molar absorptivity, α is the collection efficiency, and L is the absorption path length.

For temperature measurement in our experiment, the ratio of the two fluorescence intensities is used to calibrate temperature. The use of the ratio allows compensating for possible variations in the excitation light intensity, variations in fiber bend, photobleaching, etc. The ratio of RH110 and RHB is calculated by the following formula

$$\frac{I_{eRH110}}{I_{eRHB}} = \frac{\varepsilon_{RH110}\alpha_{RH110}\phi_{RH110}C_{RH110}}{\varepsilon_{RHB}\alpha_{RHB}\phi_{RHB}C_{RHB}}$$

It is obvious that I_0 is cancelled out, while α and ε are considered to be constant. As long as the concentrations of dyes are determined, the ratio only depends on quantum efficiencies, ϕ_{RH110} and ϕ_{RHB}.

Meanwhile, the volume and concentration of the dyes should be kept constant for precise measurement because the ratio also depends on them.

2 Experiments and results

The excitation light was transmitted through POF to excite the fluorophore, and the emitted fluorescence spectra encoding the temperature information was recorded by the spectrometer. In our experiment the temperature was varied from 25 ~ 60 ℃ with an increment of 1 ℃ by using a water bath. It took about 2 min for the sensing head to reach the preset temperature and another 3 min for stabilizing of temperature. During experiment a thermistor with a resolution of 0.1 ℃ was used as reference.

Fig. 2 shows the fluorescence spectra at several temperatures. It is observed that the emission peak of RHB is about 580 nm and that of RH110 is about 520 nm. The fluorescence intensity of RHB decreases monotonously with the rising temperature, while that of RH110 only shows some fluctuations. The fluctuations may be caused by the excitation light source whose instability is ± 1%, and the photobleaching of fluorophore. The emission peaks of RH110 and RHB are 60 nm apart and can be separated easily.

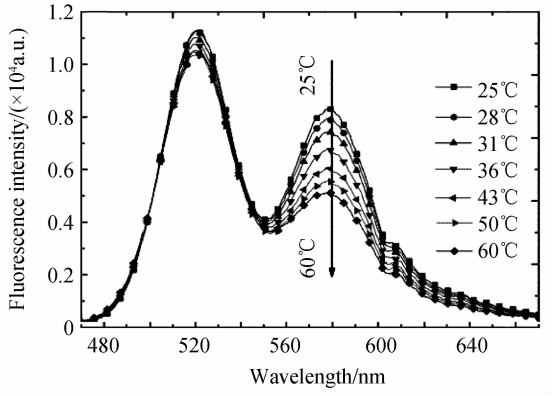


Fig. 2 The fluorescence spectra of the mixture of RHB and RH110 recorded by fiber-optic spectrometer when heating the sensing head from 25 to 60 ℃

Moreover, based on the results shown in Fig. 2, we also investigate the sensitivity of the fluorescence spectrum of RH110 and RHB to the variation of temperature as a function of wavelengths. Like other group did[12], the sensitivity of fluorescence spectrum,

which is displayed as the ratio of spectral signal at a change of temperature ΔT, $I(\lambda, T_{ref}+\Delta T)$, to that at a reference temperature, $I(\lambda, T_{ref})$, is calculated and illustrated in Fig. 3. Here, the reference temperature is set at 25 ℃. It can be seen that the most temperature-sensitive wavelength range is around the emission peak of RHB, where an average sensitivity of 1.1% K^{-1} is observed from 25～60 ℃. Thus, it is reasonable for us to choose the spectral interval around 580 nm to represent the fluorescence spectrum of RHB. As for RH110, two temperature-insensitive wavelength ranges locate at 500 nm and 545 nm, where a sensitivity of 0.06% K^{-1} and 0.22% K^{-1} can be noticed, respectively. Therefore, it prompts us to select either of these two spectral intervals to represent the spectrum of RH110.

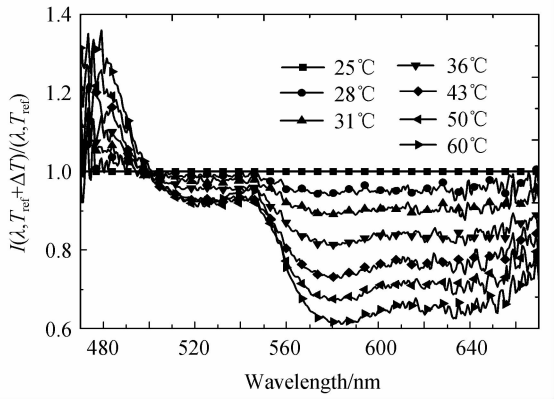
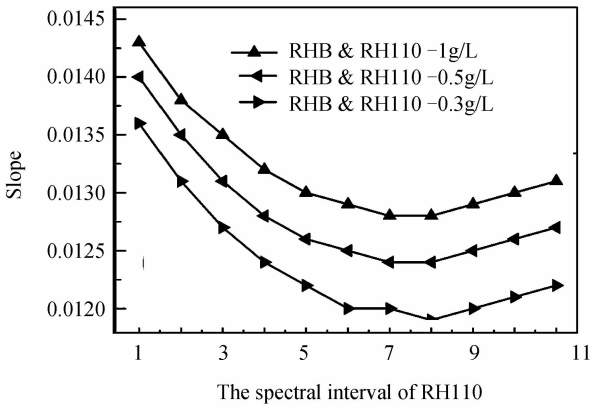


Fig. 3 Temperature dependence of the fluorescence spectra of the sensor at various temperatures is given

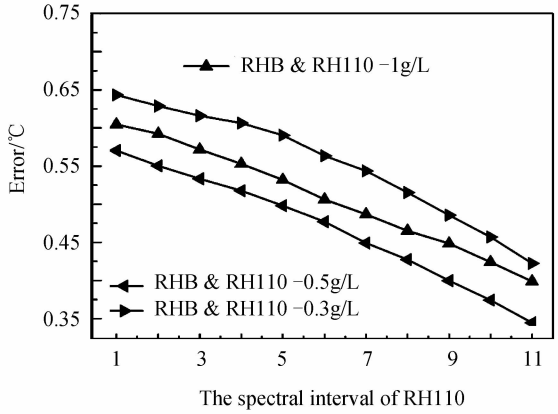
In fact, the principle of the sensor is based on the temperature-dependent ratio of two dye′s fluorescence intensities. For accurate temperature measurement the sensor system should be designed for dual wavelength operation because it would be easy to measure the ratio of the fluorescence intensities at two wavelengths as a function of temperature[21]. As there are numerous spectral regions which can be applied into fluorescence ratiometric calculation, it is important to note that with proper choice of spectral intervals, for our sensor system developed herein, the ratio of intensities would be linearly scaled to temperature in our tested range.

As is shown in Fig. 3, the most temperature-sensitive region of the spectrum is at about 580 nm, thus it is reasonable for us to choose the spectral interval of 570～590 nm to represent the fluorescence wavelength range of RHB, and the intensity integration over this range is the fluorescence signal of RHB in our experiment. On the other hand, it is also important to select a proper fluorescence wavelength range to represent the integration interval of RH110. In our experiment the wavelength 495-545 nm is considered to be the fluorescence range of RH110. In order to identify the optimal spectral interval of RH110, the interval width of RH110 is chosen to be 20 nm, 14 nm and 8 nm, respectively. When the interval is 20 nm, the corresponding spectral interval of RH110 can be set as 495～515 nm, 498～518 nm, 501～521 nm, … until 525～545 nm, respectively. It should be noted that the lower bound of each interval increases with an increment of 3 nm. Likewise, when the interval is 14 nm, the corresponding spectral interval of RH110 is set at 495～509 nm, 498～512 nm, 501～515 nm, … until 531-545 nm, and when the spectral width is 8 nm, the corresponding interval is set as 495～503 nm, 498～506 nm, 501～509 nm, … until 537～545 nm. Thus, the numbers of the selected spectral intervals of RH110 correspond to interval widths of 20 nm, 14 nm and 8 nm, are 11, 13 and 15, respectively, which are plotted as the abscissas shown in Fig. 4.

In order to illustrate the influences of different spectral interval of RH110 on our measurement, three experiments of different concentrations of dyes were conducted and corresponding spectra were acquired. With the spectral intervals of RHB and RH110 mentioned in last paragraph, the intensities ratio of RH110 and RHB can be obtained. It is found that the ratio changes linearly with temperature. Fig. 3 illustrates the slopes and rms temperature errors of the



(a) Slopes: width of 20 nm



(b) RMS error: width of 20nm

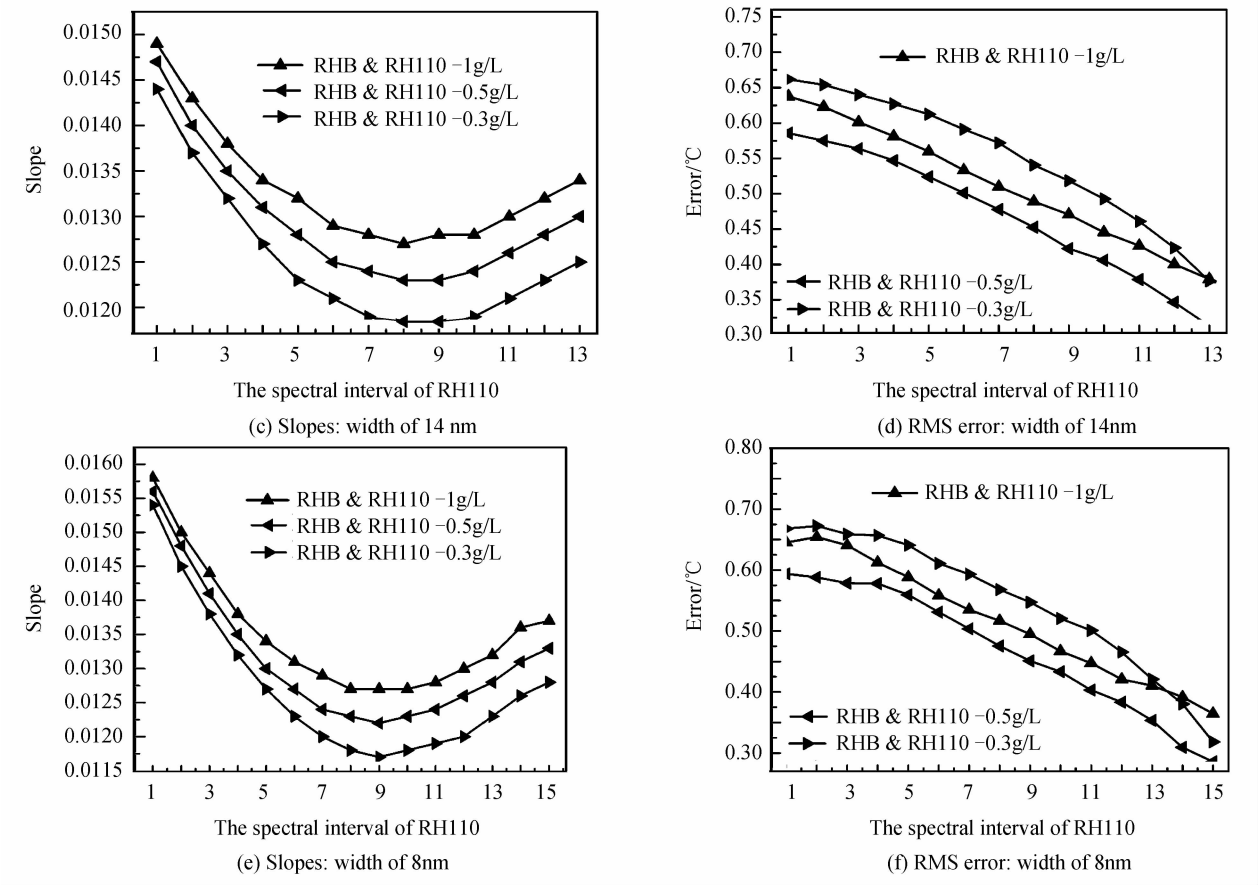


Fig. 4 Three experiments with different concentrations of RHB and RH110

linear fitted results of the ratios corresponding to different spectral intervals of RH110. From Fig. 4 (a), (c) and (e), it can be noticed that as the spectral interval of RH110 changes from around 495 nm to around 545 nm, the slopes of the linear fitted calibration curves show a decrease initially, and then rise back to a moderate value. Moreover, as the concentrations of dyes decrease from 1 g/L to 0.3 g/L, the slopes corresponding to all the intervals of RH110 diminish as well. On the other hand, from Fig. 4 (b), (c) and (d), it can be observed that the rms temperature measurement errors show a monotonous decrease as the interval moves towards 545 nm, in particular, the minimum rms temperature error of 0.28 ℃ is obtained with the spectral interval of 537～545 nm.

Therefore, it can be concluded that the optimal spectral intervals which can be used for temperature measurement by FIR method are 537 ～ 545 nm for RH110 and 570 ～ 590 nm for RHB, with a slope (sensitivity) of 0.0128 and a rms temperature error (uncertainties) of 0.28 ℃. With these optimal spectral intervals, the fluorescence intensities corresponding to three different concentrations of dyes, namely 1 g/L, 0.5 g/L, and 0.3 g/L are acquired. As shown in Fig. 5 (a) and (b), the temperature dependence of RHB is

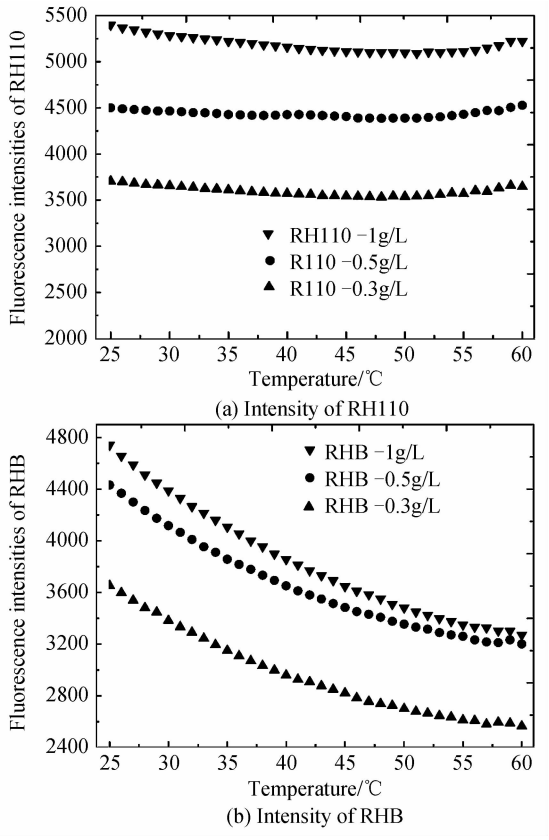


Fig. 5 The fluorescence intensities of RH110 and RHB corresponding to three different concentrations of dyes

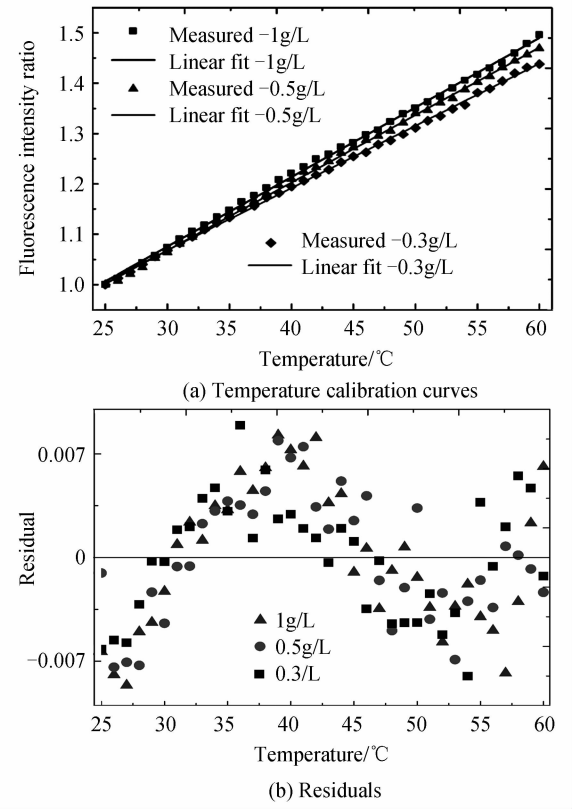


Fig. 6 The temperature calibration curves corresponding to three different concentrations of dyes and residuals to the linear fit

much stronger than that of RH110. RHB shows an average decrease of 1.1%/K while RH110 only shows some fluctuations. Moreover, the fluorescence intensities of both dyes decrease with the concentrations diminishing. Fig. 6 shows the temperature calibration curves under three concentrations, which are obtained by the fluorescence intensities in Fig. 5. The thermal response of the ratios in Fig. 6 using the linear regression can be expressed as [7]

$$r_1 = 0.0138T + 0.6732,$$
$$r_2 = 0.0134T + 0.6825,$$
$$r_3 = 0.0128T + 0.6716,$$

where r_1, r_2, and r_3 refer to the ratios obtained for the concentrations of 1 g/L, 0.5 g/L and 0.3 g/L. All the three curves in Fig. 6 show good linear property of regression, with the adjusted R-square values of 0.999, 0.999, 0.998, respectively. The rms deviations of the data are 0.004968, 0.004288, 0.003584 for the concentrations of 1 g/L, 0.5 g/L and 0.3 g/L, which results in the rms temperature errors of 0.36 ℃, 0.32 ℃, and 0.28 ℃, respectively. The temperature sensitivities for the concentrations of 1 g/L, 0.5 g/L and 0.3 g/L are 0.0138/K, 0.0134/K and 0.0128/K, which are approximately equal to the sensitivity reported in Ref. [16]. Based on these sensitivities and the residuals shown in Fig. 6, it can be concluded that the typical resolution of our sensor is 0.3 ℃.

Actually, despite the existence of fluctuations of RH110 shown in Fig. 2, they have negligible influence on the performance of our sensor because these uncertainties are eliminated by FIR[16-17,19-20], as the results illustrated in Fig. 6. Before each measurement, the self-calibration of spectrometer is conducted to remove drifting.

The stability of the sensor using fluorescence agents as temperature indicators is always a concern in many applications. The sensor of this work uses RH110 and RHB as temperature indicators. RH110 and RHB belong to xanthene group which is highly stable. In addition, the intensity of the excitation light used in this work is very low compared with pulsed lasers, which are normally utilized in fluorescence intensity based optical fiber temperature sensors. Hence there is no problem of photon-induced decomposition of the fluorescence agent of our sensor. Based on these properties, the sensor of this work is expected to possess a long lifetime. Actually, the sensor has been used for 3 months to obtain the experimental results, and the sensitivity (concentration of 0.3g/L) has been observed to increase slightly (1.6%) from 0.0128 to 0.0130. This may be caused by the lack of protection afforded to the sensor, leading to the decay of the fluorescent dye.

3 Conclusion

We proposed a dual fluorescence type optical fiber temperature sensor which could be used to detect the temperature of 25 ~ 60 ℃ by using fluorescence intensity ratiometric method. Three experiments with different concentrations of dyes were conducted and corresponding spectra were obtained, and based on this point, the dependence of the measurement results on different spectral intervals of RH110 was investigated. It was found that 537~545 nm is the optimal spectral interval for RH110 for temperature measurement in our experiment when spectral interval of RHB is set as 570~590 nm. With these optimal spectral intervals, a sensitivity of 0.0128/K (±0.003584) and corresponding rms temperature error of 0.28 ℃ are obtained. Comparing with those sensors using only one fluorescence agent, our sensor is capable of eliminating the uncertainties and drifting caused by the excitation light fluctuations and the photo-bleaching of fluorophore. Considering that the spectrometer could be replaced by a pair of photo-diodes with band-pass filters, and that the Tungsten Halogen lamp (with a band-pass filter) could be replaced by a LED, a temperature sensing device with a simple structure and low cost can be fabricated, thus it may have potential

to be applied in point or multi-point temperature measurement.

References

[1] ZHANG Ji-jun, WU Zu-tang, PAN Guo-feng, *et al*. Design of optical fiber grating-based high-precision and low frequency vibration sensor[J]. *Acta Photonica Sinica*, 2014, **43**(s1): 0128001.

[2] LIU Jian-qing, YIN Fei-fei, LI Jian-qiang, *et al*. Resolution improved Raman distributed temperature sensors based on error compensation[J]. *Acta Photonica Sinica*, 2014, **43**(s1): 0128002.

[3] LIN Xiao, REN Li-yong, XU Yi-ping, *et al*. Low-cost multipoint liquid-level sensor with plastic optical fiber[J]. *IEEE Photonics Technology Letters*, 2014, **26**(16): 1613-1616.

[4] JING Yao-qiu, JIANG Yi, XIAO Shang-hui, *et al*. A differential absorption based optical fiber Methane gas sensing system[J]. *Acta Photonica Sinica*, 2014, **43**(4): 0428002.

[5] MINARDO A, BERNINI R, ZENI L. Distributed temperature sensing in polymer optical fiber by BOFDA[J]. *IEEE Photonics Technology Letters*, 2014, **26**(4): 387-390.

[6] MIZUNO Y, NAKAMURA K. Potential of Brillouin scattering in polymer optical fiber for strain-insensitive high-accuracy temperature sensing[J]. *Optics Letters*, 2010, **35**(23): 3985-3987.

[7] LUO Yan-hua, WU Wen-xuan, WANG Tong-xin, *et al*. Analysis of multimode BDK doped POF gratings for temperature sensing[J]. *Optics Communications*, 2012, **285**(21): 4353-4358.

[8] TAO S, JAYAPRAKASH A. A fiber optic temperature sensor with an epoxy-glue membrane as a temperature indicator[J]. *Sensors and Actuators B: Chemical*, 2006, **119**(2): 615-620.

[9] DUONG H D, RHEE J I. Exploitation of thermo-effect of rhodamine B entrapped in sol-gel matrix and silica gel for temperature detection [J]. *Sensors and Actuators B: Chemical*, 2007, **124**(1): 18-23.

[10] ZHANG Z, GRATTAN K T V, PALMER A W. Fiber-optic high-temperature sensor based on the fluorescence lifetime of alexandrite[J]. *Review of Scientific Instruments*, 1992, **63**(8): 3869-3873.

[11] AIZAWA H, KATSUMATA T, KOMURO S, *et al*. Fluorescence thermometer based on the photoluminescence intensity ratio in Tb doped phosphor materials[J]. *Sensors and Actuators A: Physical*, 2006, **126**(1): 78-82.

[12] CASTRELLON-Uribe J. Experimental results of the performance of a laser fiber as a remote sensor of temperature [J]. *Optics and Lasers in Engineering*, 2005, **43**(6): 633-644.

[13] LI D, WANG Y, ZHANG X, *et al*. Optical temperature sensor through infrared excited blue upconversion emission in Tm^{3+}/Yb^{3+} codoped Y_2O_3 [J]. *Optics Communications*, 2012, **285**(7): 1925-1928.

[14] CASTRELLON J, PAEZ G, STRIJNIK M. Radiometric analysis of a fiber optic temperature sensor[J]. *Optical Engineering*, 2002, **41**(6): 1255-1261.

[15] LIU L, WANG Y, ZHANG X, *et al*. Optical thermometry through green and red upconversion emissions in $Er^{3+}/Yb^{3+}/Li^{+}$: ZrO_2 nanocrystals[J]. *Optics Communications*, 2011, **284**: 1876-1879.

[16] LAM H T, KOSTOV Y, TOLOSA L, *et al*. A high-resolution non-contact fluorescence-based temperature sensor for neonatal care[J]. *Measurement Science and Technology*, 2012, **23**(3): 035104.

[17] KUESTER T, ROBUCCI R, LAM H, *et al*. High resolution fluorescence-based temperature sensor for stand-off detection in physiological range[C]. Sensors, 2013 IEEE. IEEE, 2013: 1-4.

[18] LIN Xiao, REN Li-yong, QU En-shi, *et al*. Theoretical and experimental study on nonintrusive light injection via cladding in plastic optical fibers [J]. *Journal of Lightwave Technology*, 2013, **31**(3): 359-365.

[19] SAKAKIBARA J, ADRIAN R J. Whole field measurement of temperature in water using two-color laser induced fluorescence[J]. *Experiments in Fluids*, 1999, **26**(1-2): 7-15.

[20] CHAUHAN V M, HOPPER R H, ALI S Z, *et al*. Thermo-optical characterization of fluorescent rhodamine B based temperature-sensitive nanosensors using a CMOS MEMS micro-hotplate[J]. *Sensors and Actuators B: Chemical*, 2014, **192**: 126-133.

[21] RAI V K. Temperature sensors and optical sensors[J]. *Applied Physics B*, 2007, **88**(2): 297-303.