Exp Fluids (2008) 45:869–881
DOI 10.1007/s00348-008-0506-4

RESEARCH ARTICLE

A laser-induced fluorescence measurement for aqueous fluid flows with improved temperature sensitivity

Jeffrey A. Sutton · Brian T. Fisher · James W. Fleming

Received: 7 May 2007 / Revised: 12 February 2008 / Accepted: 3 April 2008 / Published online: 13 June 2008

Abstract This paper presents temperature-sensitive laser-induced fluorescence measurements of Fluorescein 27 dissolved in aqueous solutions. We show that Fluorescein 27, dissolved in water and excited by a 532-nm Nd:YAG laser pulse, yields improved temperature sensitivity over traditional organic dyes such as Rhodamine B. The high temperature sensitivity of Fluorescein 27 when excited at 532 nm is due primarily to a temperature-dependent shift of the absorption spectrum to longer wavelengths for increased temperatures. The linearity of the fluorescence signal with respect to the incident laser intensity and dye concentration is reported. In addition, Fluorescein 27 dissolved in an aqueous solution remains photo-stable for $>10^5$ laser pulses at both ambient and high temperatures ($T > 60$°C) when excited with low-irradiance laser pulses. Finally, we demonstrate that using a dual tracer (or ratiometric) technique in which the fluorescence from Fluorescein 27 and another dye (e.g., Rhodamine B or Kiton Red 620) are detected following the 532 nm excitation results in a significantly enhanced temperature sensitivity over a single tracer measurement and previously reported dual tracer methods. Such temperature sensitivity is useful in multi-dimensional temperature imaging and temporally resolved measurements.

J. A. Sutton · B. T. Fisher · J. W. Fleming
Navy Technology Center for Safety and Survivability,
Chemistry Division, Combustion Dynamics Section, Code 6185,
Naval Research Laboratory, 4555 Overlook Ave. SW,
Washington, DC 20375-5342, USA

Present Address:
J. A. Sutton (✉)
Department of Mechanical Engineering,
The Ohio State University,
Columbus, OH 43210, USA
e-mail: sutton.235@osu.edu

Present Address:
B. T. Fisher
Sandia National Laboratories, Livermore, CA 94551, USA

1 Introduction

Numerous studies have used laser-induced fluorescence (LIF) as a diagnostic technique to measure fluid flow scalars such as temperature or species concentrations. In particular, temperature measurements are widely made to ascertain specific information about physical processes such as natural convection (e.g., Coolen et al. 1999; Sakakibara and Adrian 2004), turbulent mixing and heat transfer in laboratory flows (e.g., Sakakibara et al. 1993; Coppeta and Rogers 1998; Lemoine et al. 1999; Rehab et al. 2000; Sakakibara and Adrian 1999; Seuntiëns et al. 2001), tribological flows (e.g., Hidrovo and Hart 2000, 2001) and evaporating or combusting droplets (e.g., Lavieille et al. 2000, 2001). The major limitations of the LIF technique for temperature measurements in aqueous fluid flows are: (1) the fluorescent tracer (typically an organic dye) must be water-soluble; (2) the fluorescent tracer must exhibit a sufficient temperature dependence over a suitable temperature range; (3) the fluorescent tracer must be photo-stable under experimental conditions that yield detectable emission (suitably high concentrations and laser intensities); (4) the absorption and emission of the dye must be spectrally separable; and (5) the re-absorption of the emitted fluorescence signal must be minimal. The fluorescence intensity for dilute mixtures with low-irradiance laser excitation can be written as:

$$I = KI_0C\varepsilon\Phi, \tag{1}$$

where K is a geometrical constant describing the collection efficiency of the optical system, I_o is the incident excitation

intensity at the point of interest, C is the concentration of the fluorescent dye, ε is the absorption coefficient, and Φ is the quantum yield. For a select number of dyes, ε and/or Φ are temperature dependent, leading to a temperature-sensitive emission and a marker of the temperature within the fluid flow. For example, a decrease of fluorescence signal of Rhodamine B (also called Rhodamine 610) with increasing temperature has been reported due to a decrease in Φ (Lopéz Arbeloa and Rohatgi-Mukherjee 1986; Lopéz Arbeloa et al. 1991), which is induced by increased quenching effects with increased temperature (e.g., Lemoine et al. 1999).

Coppeta and Rogers (1998) and Saeki and Hart (2001) conducted reviews of the most common fluorescent dyes used for LIF experiments and found that Rhodamine B has one of the highest temperature sensitivities when excited with common sources such as argon-ion (488 and 514.5 nm) and Nd:YAG (532 nm) lasers. Rhodamine B is one of the most widely used fluorescent tracers for measuring temperature in aqueous flows due to its high solubility in water, low cost, and high absorption and emission, in addition to its temperature sensitivity. The data of Coppeta and Rogers (1998) and Saeki and Hart (2001), along with that of other researchers are summarized in Table 1, yielding an average temperature sensitivity of −1.58% per °C, that is as the temperature increases, the intensity of the emitted fluorescence signal decreases.

Another dye that exhibits good solubility in water, low cost, and high quantum yield is Fluorescein 27 (FL27; also called Fluorescein 548; $C_{20}H_{10}O_5Cl$). In this work, we present results showing that when excited at 532 nm (Nd:YAG laser), the fluorescence emission of FL27 increases with increasing temperature and the temperature sensitivity is higher than that of Rhodamine B. The positive temperature dependence of FL27 when excited at 532 nm is due to a temperature-dependent absorption curve, where the absorption coefficient increases with increasing temperatures. The linearity of the fluorescence signal with respect to the incident laser energy and dye concentration is reported. In addition, previous problems associated with the use of fluorescein dyes such as "photobleaching" were not observed for the present experimental conditions using a pulsed laser. Finally, we present results from a dual tracer or "ratiometric" technique (e.g., Coppeta and Rogers 1998; Hidrovo and Hart 2000; Kim et al. 2003) in which the emitted fluorescence from FL27 (positive temperature dependence) and another suitable dye (negative temperature dependence) are detected following the 532-nm excitation. By taking the ratio of the fluorescence emissions of two fluorescent dyes with opposite temperature dependencies, a significantly enhanced temperature sensitivity is achieved over the single FL27 measurement. We examine several "red-emitting" dyes as potential ratiometric partners with FL27 and evaluate their appropriateness based on temperature sensitivity, quantum yield, and spectral separation from FL27 emission. We show that the ratio of the emitted fluorescence of FL27 and Kiton Red 620 after 532 nm excitation yields higher temperature sensitivity

Table 1 Temperature characteristics of fluorescein and red-emitting dyes

Dye	Excitation (nm)	Temperature range (°C)	Temperature dependent emission (% per °C)	Reference
FL27[a]	**532**	**20–80**	**+3.50**	**Present study**
FL[b]	514	20–60	+2.43	Coppeta and Rogers (1998)
FL[b]	488	20–60	−0.16	Coppeta and Rogers (1998)
FL[b]	488	24–46	−0.30	Walker (1987)
RhB	**532**	**20–60**	**−1.59**	**Present study**
RhB	532	25–85	−1.34	Saeki and Hart (2001)
RhB	532	20–80	−0.80	Bruchhausen et al. (2005)
RhB	514	20–60	−1.54	Coppeta and Rogers (1998)
RhB	514	24–57	−1.82	Lavieille et al. (2001)
RhB	488	15–40	−1.95	Sakakibara and Adrian (1999)
RhB	488	20–70	−1.81	Sakakibara et al. (1993)
RhB	488	15–40	−1.82	Hishida and Sakakibara (2000)
Kiton Red	**532**	**20–60**	**−1.58**	**Present study**
Kiton Red	514	20–60	−1.55	Coppeta and Rogers (1998)
Rh101	**532**	**20–60**	**−0.70**	**Present study**
Rh101	532	20–85	−0.10	Saeki and Hart (2001)

[a] FL27 (Fluorescein 27 or Fluorescein 548); $C_{20}H_{10}Cl_2O_5$

[b] Disodium fluorescein; $C_{20}H_{10}O_5Na_2$

than previously reported dual tracer methods. Such temperature sensitivity will be useful for future studies involving multi-dimensional temperature imaging and temporally resolved thermal fluctuation monitoring.

2 Experiment

2.1 Fluorescein 27

The spectroscopic properties of fluorescein dyes[1] are well known with the dyes being used in many applications ranging from dye lasers (e.g., Lindqvist 1960; Snavely 1969) to tracers in flow visualization and mixing studies (e.g., Koochesfahani and Dimotakis 1985; Koochesfahani and Dimotakis 1986; Dahm et al. 1991; Karasso and Mungal 1997). Fluorescein dyes are very attractive due to their low cost, non-toxicity, low-staining potential, high quantum yield ($\Phi > 0.9$), short fluorescence lifetime (<5 ns), water-solubility, and easy optical accessibility with commercial lasers. Figure 1 shows the absorption and emission spectrum of FL27 in an aqueous solution at 23°C. The arrow marks the location of the Nd:YAG laser (532 nm) used for excitation in the present study. It is apparent from Fig. 1 that the absorption cross-section of FL27 is very low at 532 nm compared to other common excitation wavelengths such as 488 and 514 nm. This problem is partially circumvented by the higher intensities of the pulsed Nd:YAG laser. It is noted that the peak fluorescence of FL27 is located at approximately 525 nm, which is shifted to a shorter wavelength with respect to the excitation line at 532 nm. In fact, a significant portion of the FL27 emission spectrum is anti-Stokes-shifted with respect to the excitation line. Careful filtering of both the laser-line and the anti-Stokes fluorescence may be required to accurately capture the liquid temperature due to the fact that the anti-Stokes portion of the emitted fluorescence could potentially re-absorb in the aqueous medium (optical trapping). This is discussed further in Sect. 3.

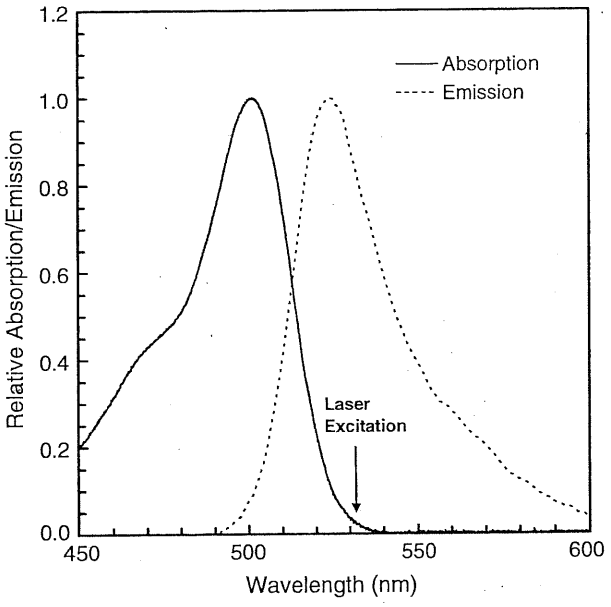

Fig. 1 Relative absorption and emission spectra of FL27 at 23°C. *Arrow* indicates excitation wavelength ($\lambda_{exc} = 532$ nm) used in the present study

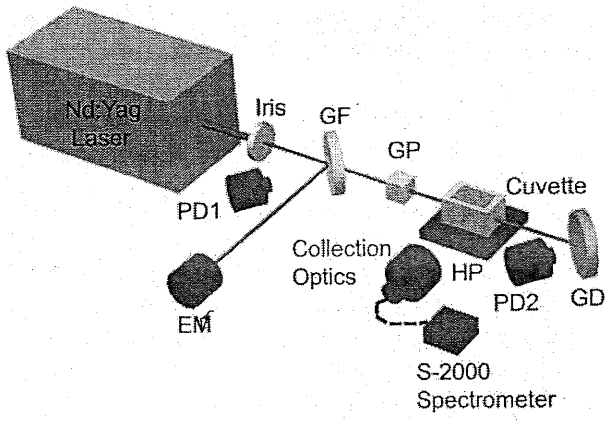

Fig. 2 Experimental setup. *PD1* and *PD2* photodiodes, *GF* glass flat, *GP* Glan Laser polarizer, *GD* glass diffuser, *EM* energy meter, *HP* hotplate. Two thermocouples (spaced 7 mm apart in all three dimensions) are imbedded in the glass cover slide of the cuvette as discussed in text

2.2 Experimental setup

The experimental setup used in the present investigation is shown in Fig. 2. The excitation source is an Nd:YAG laser (New Wave Gemini) outputting a 6-ns laser pulse at 532 nm. The laser pulse passed through a 2.3-mm-diameter iris, a 1-mm-thick glass flat (GF), and a Glan Laser single escape window polarizer (GP) before passing through a 4-cm quartz cuvette holding the aqueous solution. The 532-nm laser beam was directed through the iris to ensure that the portion of the laser beam passing through the cuvette had uniform intensity. The laser was directed through the GF to pick off a portion of the beam (~5%) that was sent to an energy meter (Molectron Energy Max 500) for average pulse energy measurements. A photodiode (PD1, rise time < 5 ns) detected the reflection off the GF to monitor the incident laser energy. This measurement is important for the temperature-dependent absorption measurements discussed later in this section. To avoid

[1] The most common fluorescein dyes are fluorescein 27 (used in this study) and disodium fluorescein.

polarization effects on the interpretation of the collected fluorescence emission, the laser passed through the Glan Laser polarizer to ensure that only vertically polarized laser light passed through the cuvette. The emitted fluorescence was collected and focused through a collection system consisting of two spherical lenses (f/4) onto a fiber optic spectrometer (Ocean Optics S2000) which is sensitive in the ultraviolet through the near infrared with a spectral resolution of 0.6 nm. The collimating lenses and fiber optic coupler were mounted on an X–Y translation stage for optimal alignment. In order to synchronize the spectrometer acquisition to the Nd:YAG laser pulse, a trigger pulse from the laser flashlamp was sent to a pulse generator (Stanford Research Systems DG535). In turn, the pulse generator sent a well-conditioned trigger pulse to the spectrometer such that an emission spectrum was collected for each laser pulse. Each fluorescence spectrum presented is the average of 500–1,000 laser pulses. In this way, the influences of laser intensity fluctuations are minimal. This is verified with the average energy measurements; however any small differences in the laser intensities between spectra were still corrected using the average energy measurements. The typical shot-to-shot variation in energy was less than 10%, and the average intensity difference between data sets (single average spectrum) was less than 2%.

In order to measure the temperature dependence of FL27, the quartz cuvette containing the fluorescein/water solution was mounted to a Corning hotplate (HP) and heated slowly (approximately 0.25°C/min). The cuvette was filled completely with the aqueous solution and sealed from the surrounding environment by a glass cover slide that used the adhesive forces between the liquid and the glass to hold the glass cover firmly in place. This cover slide prevents evaporation of the water and an inadvertent increase in the FL27 concentration and fluorescence signal as the temperature was increased. Thermal expansion and the corresponding change in concentration as the temperature increases at the measurement volume were also accounted for, even though the effects are minimal. For example, if we assume that the density of the FL27/water mixture can be represented by the density of water alone, over the temperature range of 20–100°C, the density of FL27/water mixture decreases by less than 4% (Lide 1990), leading to a concentration change of FL27 at the measurement volume of less than 0.05% per °C. The temperature was measured by two one-millimeter-diameter-type-K thermocouples and outputted to an Omega DP462 multi-channel readout. The thermocouples were inserted through the glass cover slide and permanently affixed to the slide, with the insertion holes sealed. The two thermocouples are offset from each other by 7 mm in all three coordinate planes. The exciting laser pulse traverses in the region between the two thermocouples; therefore, the average of the two temperature readings defines an average temperature in the measurement volume. For all measurements, the two temperature readings did not vary by more than 0.1°C, indicating a uniformly heated measurement volume.

Solutions of FL27 and water were made at concentrations ranging from 1×10^{-8} to 1×10^{-5} mol/L. Each solution was mixed using a magnetic stirrer and the fluorescein was allowed to dissolve in the water for a period of 1 week. The final concentration was verified by performing absorption measurements with a UV-VIS spectrophotometer (Hitatchi U-3000) and calculating the concentration at 490 nm from the Beer–Lambert Law using an absorption coefficient of 8.8×10^4 L mol^{-1} cm^{-1} (Lindqvist 1960; Bowers and Porter 1967). For the ratiometric measurements discussed in Sect. 3.4, a series of red-emitting dyes (see Table 1) were mixed in aqueous solutions similar to the FL27/water mixtures and evaluated for their temperature properties. When measuring the temperature dependence of these "red" dyes, the molar concentration of the dyes was less than 1×10^{-7} mol/L. For all ratio measurements (FL27 and an additional red dye mixed with water), the concentration of FL27 was 6×10^{-7} mol/L and the concentration of the red dye was 9×10^{-8} mol/L. These concentrations resulted in less than 4% absorption of the laser beam through the 4-cm cuvette.

In addition to the fluorescence measurements as a function of temperature, relative absorption measurements at 532 nm were made as a function of temperature. After passing through the cuvette containing the FL27/water solution, the 532 nm laser beam was directed to a glass diffuser (GD) and the transmitted laser intensity was monitored by a second fast photo-diode (PD2) and normalized by the monitored intensity from PD1 (see Fig. 2). The signals were recorded on a digital oscilloscope (Infiniium 54845A, Agilent Technologies) having a digitization resolution of 250 ps. For each temperature, 1,000 individual waveforms were used to calculate the transmitted portion of the laser. The absorption at a given temperature is then calculated as $1 - I/I_o$, where I is the transmitted laser intensity and I_o is the incident laser intensity. These relative absorption measurements as a function of temperature were put on an absolute scale by measuring the absorption coefficient at room temperature. The absorption coefficient at 532 nm was measured by holding the temperature constant ($T = 23$°C) and measuring the laser absorption as the absorption pathlength was varied from 1 to 4 cm. The absorption coefficient was calculated from the Beer–Lambert relation.

3 Results

3.1 Fluorescence response

There are only a few studies that report the emission response of fluorescein dyes with respect to incident laser intensity and dye concentration for excitation at 532 nm. Therefore, tests were performed to test the linearity of the present system with respect to these factors. The response of the emitted fluorescence with respect to incident laser intensity is shown in Fig. 3. For these measurements, a very dilute mixture of Fluorescein 27 in water (5×10^{-7} mol/L) was used so that absorption of the laser through the cuvette was minimal (<1%). The results presented in Fig. 3 show a linear response for laser intensities up to approximately 3×10^5 W/cm^2, where the linewidth of the Nd:YAG is ~ 1 cm^{-1}, according to the manufacturer's specifications. To ensure that all remaining measurements were performed in a region such that there is a linear response from the fluorescence signal with respect to the incident laser intensity, laser intensities of 2×10^5 W/cm^2 (i.e., laser pulse energies of 150 μJ/pulse) were used.

Figure 4 shows the response of the emitted fluorescence as a function of dye concentration in mol/L. As dye concentrations are increased, absorption of the laser along the cuvette may become important. Using a measured extinction coefficient of 3,180 L mol^{-1} cm^{-1} at $T = 23.8$°C and 532 nm, the absorption along the cell is accounted for in the final fluorescence measurements. At the highest concentration studied, the correction due to absorption was only 7% over the 4-cm pathlength. For concentrations used in the temperature-dependent measurements presented in Sect. 3.3, the correction due to absorption was less than 1%. The results in Fig. 4 show that there is a linear response for concentrations up to 2×10^{-6} mol/L, which is in agreement with previous results of Rehab et al. (2000) using a continuous argon ion laser (488 nm), but over ten times less than the results presented by Karasso and Mungal (1997) using sodium fluorescein in water with a pulsed Nd:YAG laser (532 nm).

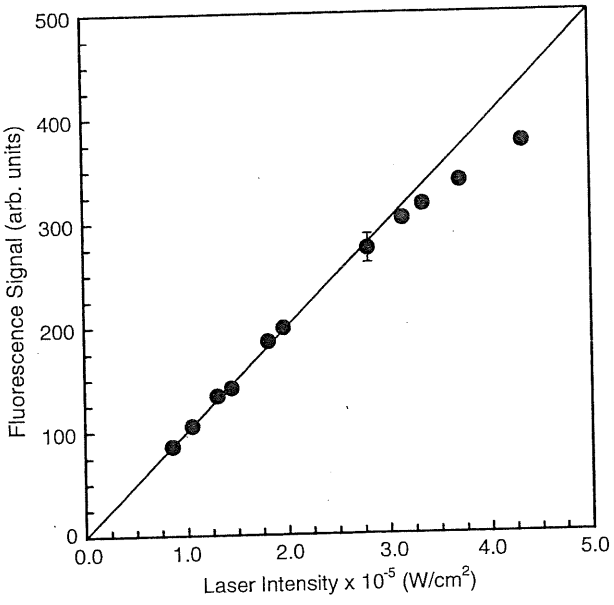

Fig. 3 Fluorescence signal versus incident laser intensity for FL27 in water ($C = 5 \times 10^{-7}$ mol/L). Excitation wavelength is 532 nm (linewidth ≈ 1 cm^{-1}). There is a linear response for laser intensities up to $\sim 3 \times 10^5$ W/cm^2. *Error bar* indicates the typical uncertainty for all measurements

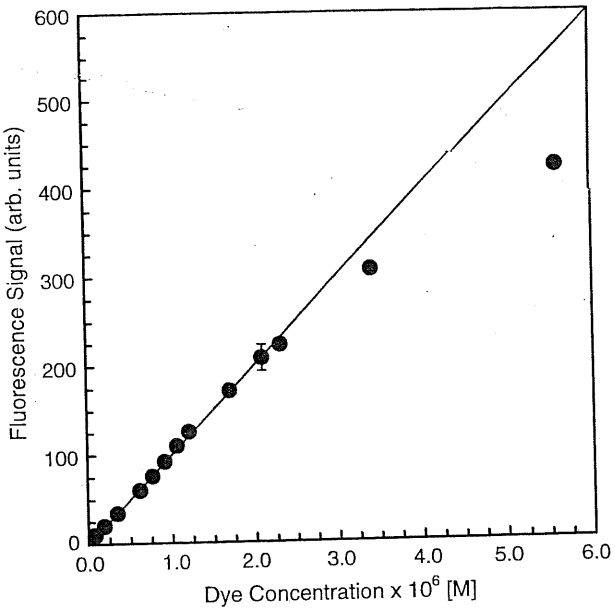

Fig. 4 Fluorescence signal versus FL27 dye concentration in water (laser intensity $= 2 \times 10^5$ W/cm^2). There is a linear response for dye concentrations up to $\sim 2 \times 10^{-6}$ mol/L. Error bar indicates the typical uncertainty for all measurements

3.2 Photo-stability

A common problem quoted in the literature about using fluorescein for quantitative purposes is its lack of photostability or problems due to "photobleaching" (e.g., Arcoumanis et al. 1990; Saylor 1995; Crimaldi 1997; Wang and Fieldler 2000; Larson and Crimaldi 2006). Photobleaching refers to the decay of the emission of light intensity over a period of time due to photodecomposition or collisional quenching of the dye. Typically, if exciting laser intensities are high enough or the dye is exposed to the exciting laser for a significant period of time, photobleaching can occur. For example, Arcoumanis et al. (1990) showed that when fluorescein in water was continually excited at 488 nm, the emitted fluorescence decreased as a

function of time and higher laser powers increased the rate of deterioration. Saylor (1995) presented results where fluorescein in water was excited using both a continuous argon-ion laser source and a "pulsed" source, which consisted of chopping the argon-ion at 1,100 Hz and consequently producing a 0.45 ms pulse. The results showed that the photobleaching effects were less pronounced using the "pulsed" source, suggesting photobleaching "recovery" during the pulsed mode runs. Saylor (1995) recommended using pulse times much less than characteristic photobleaching times, which are in the millisecond time scale (e.g., Saylor 1995; Wang and Fieldler 2000), to minimize photobleaching effects. Although Nd:YAG lasers have instantaneous energy densities that are much higher than continuous wave sources, they also have pulse lengths on the order of a few nanoseconds, which are approximately 10^5 times less than previously measured photobleaching time scales. Karasso and Mungal (1997) exposed dichlorofluorescein in water to the 532-nm pulse of a Nd:YAG laser for 1 min continuously (~600 laser pulses) and did not detect any measurable signal differences. This implied that photobleaching was not a concern under their conditions.

We continuously exposed a non-agitated (still) FL27/water solution to the exciting 532 nm laser pulse for over 2.5 h at a repetition rate of 10 Hz (10^5 laser pulses). The photo-stability results are presented in Fig. 5 for conditions at room temperature ($T = 23$°C) and at an elevated temperature of 67°C. The fluorescence signals at each time are normalized by the mean fluorescence signal over all runs. It is apparent from Fig. 5 that no decrease in emitted fluorescence signal is detectable over the course of 10^5 pulses. This implies that for the present test conditions, the dye is stable and not subject to photobleaching effects. The still dye solution is a worst-case condition because typical experiments using FL27 as a tracer for scalar measurements would be performed in a flowing system, where each local volume of dye would be exposed to a few pulses at most. For similar laser pulse energies and dye concentrations, photobleaching effects would not be important for flow visualization studies using FL27 in water and excited at 532 nm.

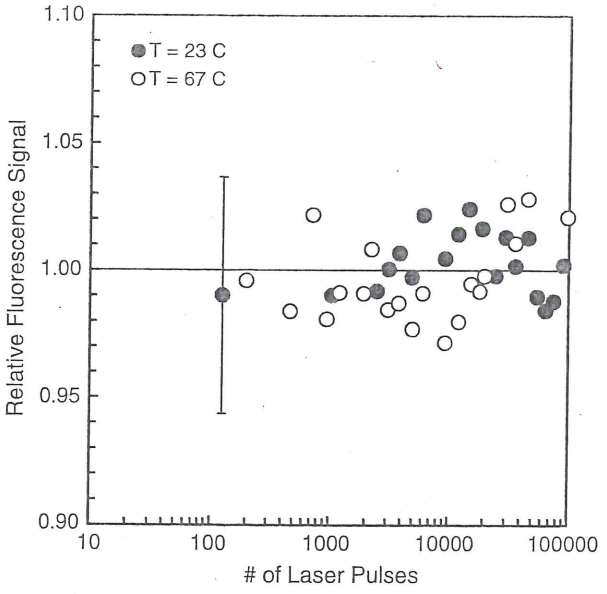

Fig. 5 Fluorescence response of FL27 in water to pulsed 532 nm (Nd:YAG) versus time (number of laser pulses). Laser intensity = 2×10^5 W/cm^2; $C = 5 \times 10^{-7}$ mol/L. *Error bar* indicates the typical uncertainty for all measurements

3.3 Temperature effects

In a recent paper by Coppeta and Rogers (1998), the temperature sensitivity of disodium fluorescein was measured when excited with a continuous argon-ion laser over a temperature range of 20–60°C. They showed that temperature effects on emitted fluorescence intensity were wavelength dependent, namely the temperature sensitivity increased from −0.16 to +2.43% per °C when the excitation wavelength was increased from 488 to 514.5 nm. It was also shown that the absorption spectrum of fluorescein is temperature dependent, shifting slightly to longer wavelengths with increasing temperature. This attribute is partially responsible for the increase in temperature sensitivity when changing the excitation wavelength from 488 to 514.5 nm and may yield higher temperature sensitivities in the emitted fluorescence when excited at 532 nm. Fluorescence spectra of FL27 in aqueous solution ($C = 5.92 \times 10^{-7}$ mol/L) excited at 532 nm for temperatures ranging from 24 to 84°C are presented in Fig. 6. Figure 6 shows that higher temperatures yield overall higher fluorescence emissions, which is consistent with the results of Coppeta and Rogers (1998) for fluorescein with excitation at 514.5 nm. As seen in Fig. 6, the emission spectrum of fluorescein shifts to longer wavelengths with increasing temperature, which is consistent with the temperature-dependent absorption spectrum shift shown by Coppeta and Rogers (1998). This temperature-dependent shift is shown more clearly in the insert in Fig. 6, which shows the fluorescence emission for 24, 52, and 84°C, normalized by the maximum for each temperature. The total shift in the peak fluorescence was 5 nm over the 60°-temperature range or approximately 0.08 nm per °C. Analysis of the data in Fig. 6 also shows that there is a small temperature-dependent broadening of the Stokes-shifted portion of the spectra with increased temperature. Integrating the emission curves (Fig. 6) from 488 to 620 nm yields the relative emission as a function of temperature for an excitation wavelength of 532 nm. Figure 7 shows that

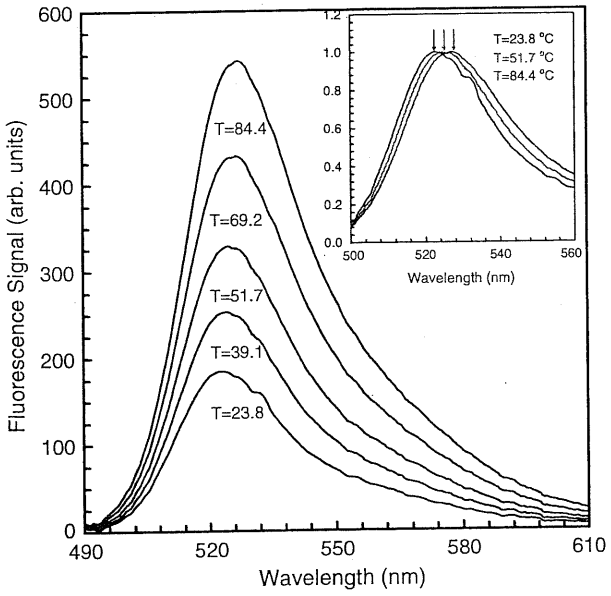

Fig. 6 Relative emission spectra of FL27 in water for temperatures ranging from 24 to 84°C. Laser intensity = 2×10^5 W/cm^2; $C = 5 \times 10^{-7}$ mol/L. *Insert* emission spectra for 24, 52, and 84°C, normalized by their respective maximum to show the temperature shift clearly. *Arrows* indicate location of peak emission

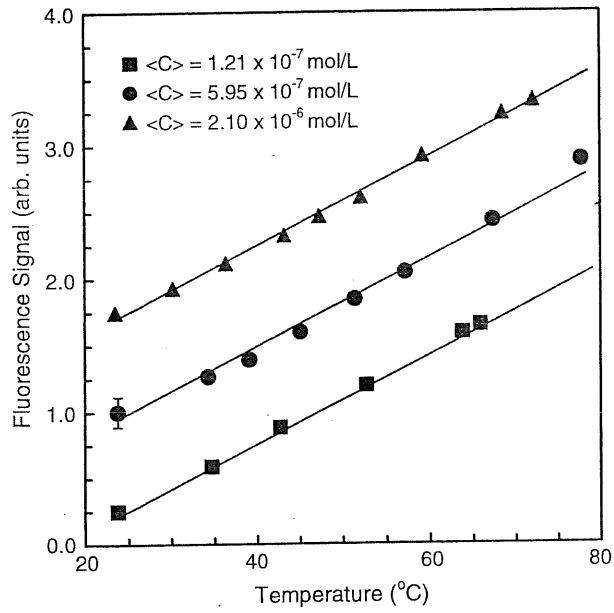

Fig. 7 Fluorescence emission versus temperature for FL27. For each concentration, the results are normalized by the fluorescence at 22°C and offset for clarity. The resultant temperature sensitivity is +3.50% per °C. For the range of conditions considered, the temperature-dependence is independent of concentration for FL27

for three different dye concentrations ranging from 1×10^{-7} to 2×10^{-6} mol/L the fluorescence intensity increases by 3.50% per °C over a temperature range of 20–80°C. Over this temperature and concentration range, the temperature dependence appears to be linear and independent of concentration. Note that the three temperature-dependent curves have been normalized by their respective fluorescence signal at 22°C and offset from each other for clarity. The concentration of the dye solution was measured both immediately before and after the experiment using absorption methods outlined in Sect. 2.2 to check for evaporation of water. The concentration measured after the high temperature experiment agreed with the initial concentration to within ±0.5%, showing evaporation was not occurring and the temperature dependence was genuine.

As mentioned in Sect. 1, the temperature dependence of the emitted fluorescence is due to either a temperature dependence of the quantum yield, Φ (e.g., Rhodamine B) or the absorption coefficient, ε. Since the quantum yield is greater than 0.9 at room temperature, it is unlikely that the positive temperature dependence of the fluorescence signal of fluorescein is due to increased quantum yield effects. Therefore, the relative absorption (absorption coefficient) was measured as a function of temperature as outlined in Sect. 2.2. An increase in ε at 532 nm with increasing temperature would be consistent with a temperature-dependent shift of the absorption curve to longer wavelengths and a slight broadening as noted by Coppeta and Rogers (1998). The relative absorption coefficients as a function of temperature are shown in Fig. 8, where the temperature-dependent absorption coefficient $\varepsilon(T)$ is calculated from the Beer–Lambert relation:

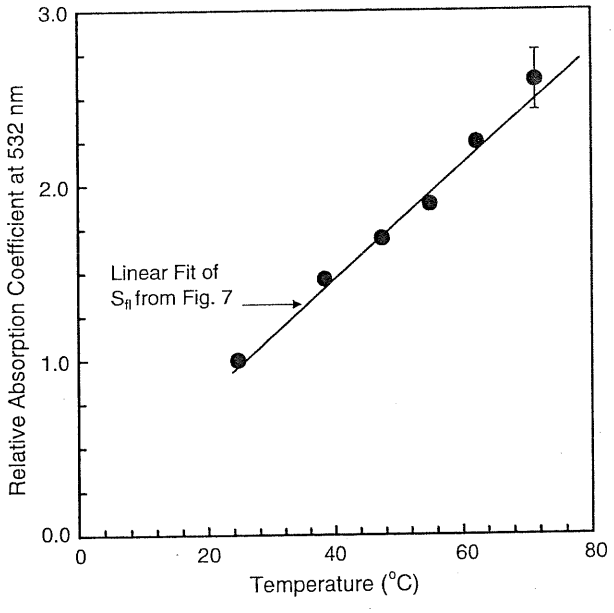

Fig. 8 Relative absorption coefficient of FL27 in water at 532 nm as a function of temperature. Also shown is the relative fluorescence (S_{fl}) as a function of temperature from Fig. 7. *Error bar* indicates the typical uncertainty for all absorption measurements

$$\varepsilon(T) = -\ln\frac{I(T)}{I_o}(Cl)^{-1}. \quad (2)$$

$I(T)$ is the transmitted laser intensity as a function of temperature, C is the concentration of the Fluorescein 27 dye ($C = 1.5 \times 10^{-5}$ mol/L) and l is the pathlength through the cuvette (4 cm). Equation (2) is valid for low concentrations where the portion of the laser light absorbed (I/I_o) is linearly proportional to both the concentration and the pathlength. The absorption coefficients at all temperatures are normalized by ε at 23.8°C, which was measured to be 3,180 L mol^{-1} cm^{-1}. Also shown in Fig. 8 is the linear fit of the relative fluorescence as a function of temperature from Fig. 7. It is clearly seen that the increase in fluorescence is linearly proportional to the increase in absorption coefficient. Therefore, the increase in FL27 fluorescence with increasing temperature is due to a temperature-dependent absorption curve as noted by Coppeta and Rogers (1998) using a continuous wavelength argon-ion laser, and not to increased values of Φ. Assuming that the broadening of the absorption curve is equivalent to the broadening observed in the emission spectra, the results in Fig. 8 imply a 0.08-nm shift in the absorption curve per °C, which is identical to the measured shift in the emitted fluorescence as discussed earlier. Using these results, we estimate that 80% of the increase in the absorption coefficient with increasing temperature is due to the temperature-dependent shift in the absorption curve.

Temperature measurements using FL27 must be made under "optically thin" conditions due to the fact that the temperature dependence of FL27 originates from a temperature-dependent absorption coefficient and the temperature may be changing in the direction of observation (Hidrovo and Hart 2001). Under optically thin conditions, the attenuation of the emitted fluorescence due to re-absorption is negligible; therefore, the effects of any temperature variation in the direction of observation on the interpretation of the fluorescence signal are not substantial, and an accurate temperature measurement can be obtained (Hidrovo and Hart 2000). We can assess the degree of optical trapping of the anti-Stokes fluorescence using the results in Fig. 8. If re-absorption of the emitted fluorescence were occurring, it would become more apparent with increasing temperature due to a greater overlap between the absorption and emission spectrums. As the temperature increases, the emitted fluorescence for wavelengths shorter than the excitation wavelength of 532 nm would not increase at the same rate as the emitted fluorescence for wavelengths longer than 532 nm, causing a change in the spectral distribution of the emitted fluorescence. However, analysis of the data in Fig. 6 indicates that the spectral distribution of the anti-Stokes portions of the emission curves are not changing (with the exception of the temperature-dependent shift) with increasing temperature; therefore, optical trapping of the fluorescence signal does not appear to be of consequence for the dye concentrations used in the present experiment.

3.4 Ratiometric technique

Previous authors have proposed a ratiometric technique (e.g., Coppeta and Rogers 1998; Hidrovo and Hart 2000; Kim et al. 2003) for increased temperature sensitivity, which is mixing two dyes with different temperature dependencies and taking the ratio of emitted fluorescence to determine temperature. Advantages of this technique not only include potential increased temperature sensitivity, but a cancellation of errors due to laser intensity fluctuations, concentration fluctuations, and extraneous laser light scattering from surfaces. All of these advantages are particularly desirable, for example, when attempting to measure spatially resolved multi-dimensional temperature fields or temporally resolved thermal fluctuations. Typical disadvantages include interferences of dye A on dye B (and vice versa) including re-absorption of the emitted fluorescence of dye A by dye B and detection of the emitted fluorescence of dye A on the spectral band of dye B.

We considered several dye candidates as ratiometric partners with FL27. In general our results showed that oxazine dyes (e.g., oxazine 170, cresyl violet) demonstrated dimerization for moderate concentrations, which is consistent with previous studies (e.g., Isak and Eyring 1992). The formation of dimers can affect the quantum yield of the dye and thus interfere with the accuracy of the temperature-based measurements. In general, xanthene dyes (e.g., rhodamine dyes) are stable, many are accessible with the 532-nm output of a Nd:YAG laser, and many exhibit temperature-dependent fluorescence emission. In this study, we examined three xanthene dyes which exhibit a negative temperature dependence as potential ratiometric partners with FL27, Rhodamine B (RhB), Kiton Red 620 (KR), and Rhodamine 101 (Rh101). Figure 9 shows the overlap of both the absorption and emission profiles of these three dyes with the FL27 emission at $T = 23$°C. Figure 9a shows that there is a significant overlap of the FL27 emission spectrum with the absorption spectrum of RhB, and decreasing overlap with KR and Rh101. Clearly, there is the potential for re-absorption of the emitted FL27 fluorescence signal when using these dyes, especially for a FL27/RhB mixture. Using the measured absorption and emission spectra and the absorptivity data of Brackmann (2000), it is estimated that less than 0.5, 0.4, and 0.3% of the FL27 fluorescence is absorbed by RhB, KR, and Rh101, respectively, for the experimental conditions (concentrations and pathlength) considered in the present study. The absorption of the RhB, KR, and Rh101 emission

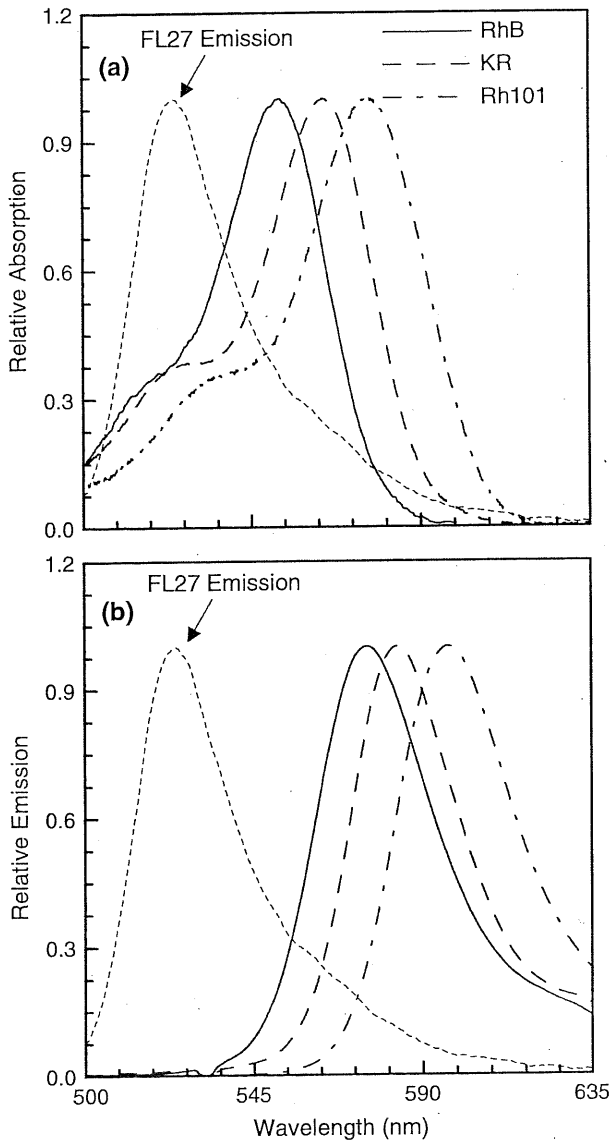

Fig. 9 Relative absorption (**a**) and emission (**b**) spectra of Rhodamine B (*RhB*), Kiton Red 620 (*KR*), and Rhodamine 101 (*Rh101*) at 23°C. Also shown in the relative fluorescence emission of FL27 at 23°C

by FL27 is negligible. Figure 9b shows that there is also an overlap between the fluorescence emission of FL27 and the emission of the three red dyes. This overlap may reduce the temperature sensitivity of the ratiometric measurement, requiring careful spectral filtering of the emitted fluorescence to ensure minimal emission overlap of the two dyes within a given detection bandpass as discussed below. Ideally, greater spectral separation between the two emission spectra results in a more convenient experimental setup, increased signal collection due to larger spectral bandwidth filters for each dye emission, and the potential for greater temperature sensitivities.

Figure 10 shows the fluorescence spectra of the three rhodamine dyes in aqueous solution excited at 532 for temperatures ranging from 22 to 80°C. As noted within the literature, increased temperatures result in decreased fluorescence emissions for the rhodamine dyes. This decreased fluorescence emission with increasing temperature makes these dyes excellent candidates as ratiometric partners with FL27, which showed an increase in fluorescence emission with increasing temperature. Integrating the emission curves shown in Fig. 10 from 540 to 660 nm yields the relative fluorescence emission as a function of temperature as shown in Fig. 11. Over the temperature range of 20–60°C, the results show decreases in fluorescence intensity of 1.59, 1.58, and 0.70% per °C for RhB, KR, and Rh101, respectively. The measured temperature sensitivities of the red dyes are in excellent agreement with previous studies shown in Table 1, giving confidence in both the rhodamine dye and FL27 results. Although the fluorescence emission of Rh101 has the greatest spectral separation from the emission of FL27 (see Figs. 9, 10), both RhB and KR have substantially higher temperature dependencies than Rh101; we only consider RhB and KR as potential ratiometric partners for the remainder of the discussion.

Figure 12 shows fluorescence spectra of FL27/RhB and FL27/KR mixtures for temperatures ranging from ~22 to 78°C. The emission of the RhB or KR occurs at longer wavelengths than that of FL27, so spectral discrimination between the two emissions is possible. For each dye combination, the portions of the spectra corresponding to FL27 emission increases in intensity for increasing temperatures, while the portions of the spectra corresponding to RhB or KR emission is decreasing with increasing temperature. It is noted that the overlap of the FL27 emission with the RhB or KR emission results in a decreased temperature sensitivity of the "red" portion of the spectrum when compared to the case of the RhB or KR alone (Fig. 10).

To evaluate the potential of the ratiometric technique in aqueous flow measurements, we applied filter masks to the results shown in Fig. 12, corresponding to the yellow fluorescence of FL27 and the red fluorescence of RhB or KR. The filter masks shown in Fig. 12 correspond to measured single-band filter data of two commercially available bandpass filters (FF01-528/38; FF01-588/21; Semrock; http://www.semrock.com). If necessary, the region around 532 nm can be filtered with a very narrow notch filter such as the NF01-532 from Semrock. As an example, this filter provides laser blocking greater than 6 OD with only a 15 nm bandpass centered at 532 nm. Therefore, fluorescence from FL27 can be easily separated from 532-nm interference. Convolving these filter masks with the spectral data shown in Fig. 12, integrating the yellow and red portions of the resulting spectra over appropriate bandwidths, and taking the ratio of the yellow

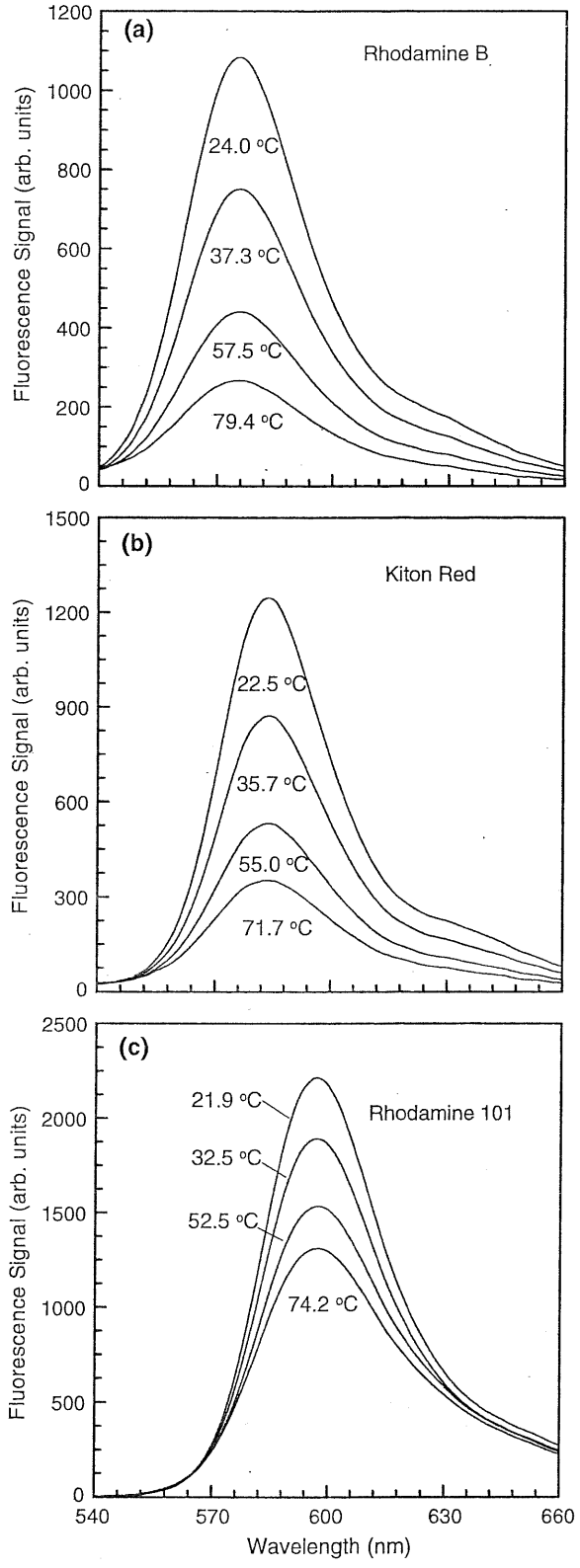

Fig. 10 Relative emission spectra of RhB (**a**), KR (**b**), and Rh101 (**c**) in water for temperatures ranging from 24 to 79°C

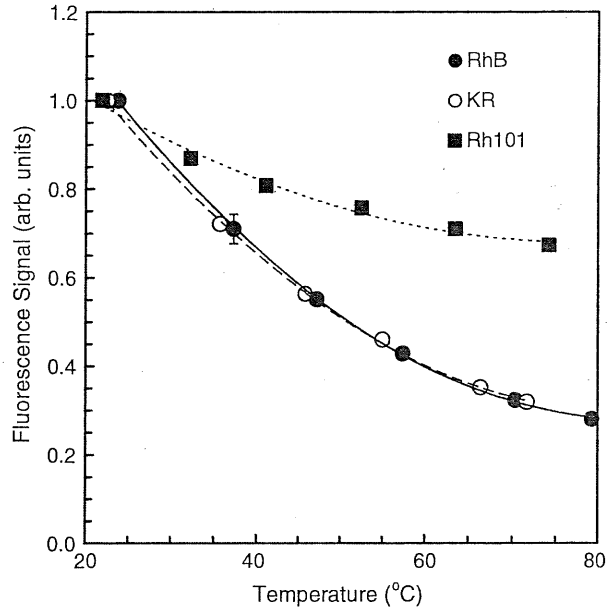

Fig. 11 Fluorescence emission versus temperature for RhB, KR, and Rh101. The temperature sensitivites are −1.59, −1.58, and −0.7% per °C, respectively

and red portions of the spectra, yields a highly temperature-dependent measurement as shown in Fig. 13. The fluorescence ratio between the two spectral regions shown in Fig. 13 was normalized by the ratio at 22°C. Using these filtering parameters maximizes the total collected fluorescence signal in each bandwidth and the fluorescence ratio of FL27 to RhB or KR, while minimizing the fluorescence interference of FL27 emission in the red dye spectral bandwidth and vice versa. For the filter conditions described above, the fluorescence ratio of FL27 to RhB or KR increases by more than a factor of four over the 20–80°C temperature range. At 22°C, 9% of the total fluorescence collected in the red dye bandwidth corresponds to that of FL27 and 7% of the total fluorescence collected in the FL27 bandwidth corresponds to that of the red dyes. At the highest temperatures considered (~80°C), the portion of the collected fluorescence in the FL27 bandwidth corresponding to the red dyes decreases to 1%, while the portion of the collected fluorescence in the red dye bandwidth corresponding to FL27 increases to 57%.

Since the fluorescence ratio is not linear with increasing temperature, the temperature sensitivity of the measurement is not constant and is a function of temperature. We fit the experimental results shown in Fig. 13 with a third-order polynomial ($R^2 = 0.9999$) and differentiated the curve-fit to estimate the temperature sensitivity over the entire temperature range. As shown in Fig. 14, the temperature sensitivity is greater than 4.5% per °C for all

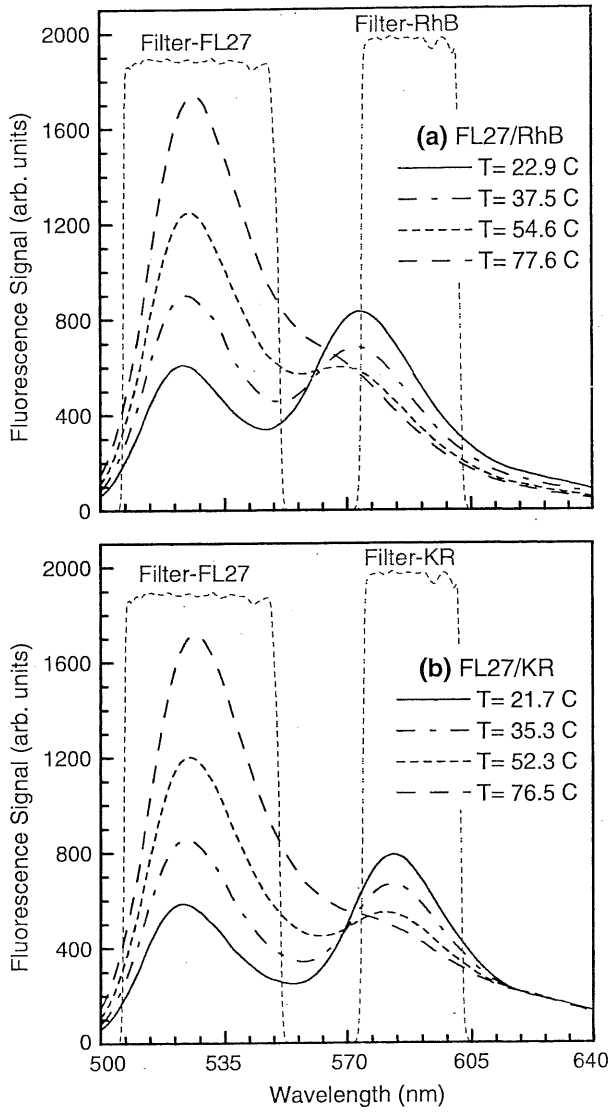

Fig. 12 Relative emission spectra of FL27/RhB mixtures (**a**) and FL27/KR mixtures (**b**) in water for temperatures ranging from 22 to 78°C. Also shown are the "filter masks" used for evaluating the ratiometric technique

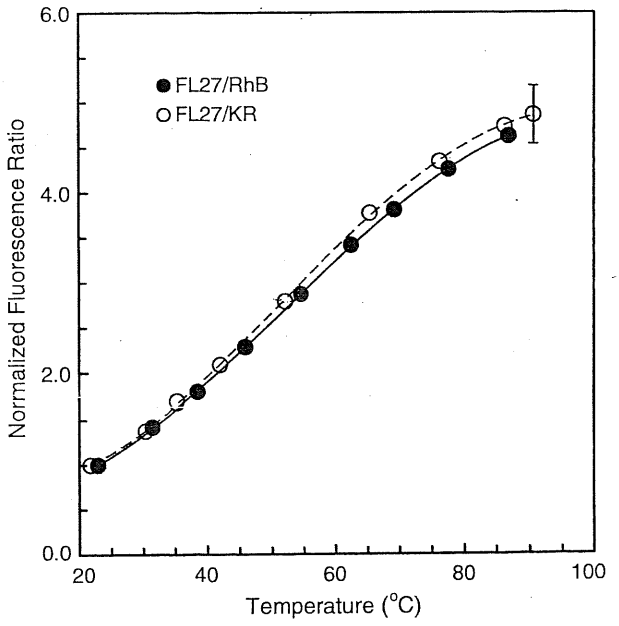

Fig. 13 Normalized fluorescence ratios as a function of temperature. The fluorescence ratio is defined as the ratio of the emitted fluorescence of FL27 to the emitted fluorescence of RhB or KR. The fluorescence ratios are normalized by the fluorescence ratio at 22°C. *Error bar* indicates the typical uncertainty for all measurements

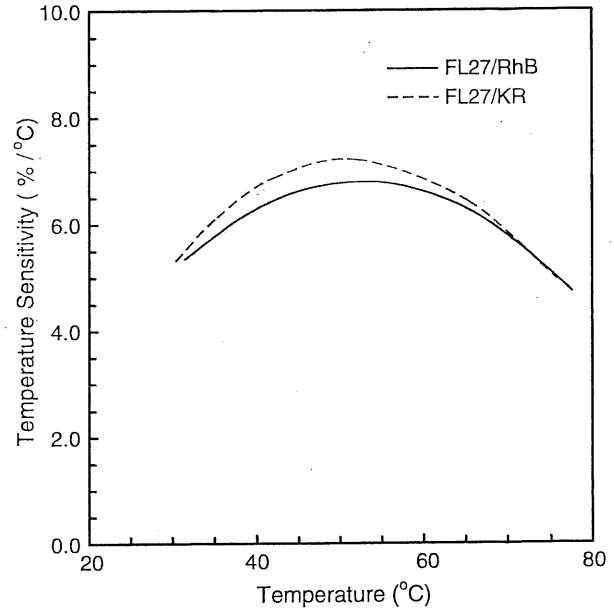

Fig. 14 Estimated temperature sensitivities using the FL27/RhB and FL27/KR mixtures and a ratiometric technique. Improved temperature sensitivity is achieved using the proposed ratiometric technique compared to previous reports in the literature

temperatures considered. The peak temperature sensitivity of the FL27/KR combination is greater than 7% per °C, while the peak sensitivity for the FL27/RhB combination is greater than 6.5% per °C. Both of these temperature sensitivities are greater than that of the single FL27 measurement shown in Sect. 3.3 and previous demonstrations of the ratiometric technique (e.g., Coppeta and Rogers 1998; Hidrovo and Hart 2000; Kim et al. 2003). The small increased temperature sensitivity when using the FL27/KR mixture over the FL27/RhB mixture is due to the increased spectral separation between the FL27 emission and the KR emission compared to the spectral separation between the FL27 and RhB emission. Since the emission of KR is shifted to longer wavelengths than RhB, there is less interference from the FL27 emission. The fluorescence intensity in the red portion of the spectra (~570 to

605 nm) also decreases at a greater rate as a function of temperature for the FL27/KR mixture when compared to the FL27/RhB mixture. It should be noted that the fluorescence emission of KR is pH independent (Coppeta and Rogers 1998), therefore the use of KR is advantageous over that of RhB when both temperature and pH vary. This does not imply that the FL27/KR mixture is useful in deducing pH variations, only that the FL27/KR mixture will not be subject to errors caused by variations in pH as would be the case of the FL27/RhB mixture.

4 Conclusions

We present an improved temperature-sensitive LIF measurement in aqueous fluid flows using Fluorescein 27 (FL27) with excitation by a Nd:YAG laser at 532 nm. The increased temperature sensitivity of FL27 (+3.50% per °C) over the more conventional Rhodamine B (−1.6% per °C) will prove beneficial in flow visualization studies or other applications in which greater temperature sensitivity is needed without a loss of signal. The increase in the emitted fluorescence of FL27 for increasing temperatures when excited at 532 nm was shown to be primarily due to a temperature-dependent shift in the absorption spectrum. Since 532 nm is located in the "red portion" of the absorption spectrum tail, a small shift in wavelength (along with a small broadening of the absorption curve) increases the absorption coefficient by almost a factor of 3 over a 60° temperature range, thus increasing the emitted fluorescence. Even though the absorption coefficient greatly increases as temperature increases, it remains small enough ($\varepsilon \approx 8{,}400$ L mol^{-1} cm^{-1} at 70°C) such that absorption of the incident laser is negligible for concentrations used in the present study ($C \approx 5 \times 10^{-7}$ mol/L). Photobleaching effects, a typical disadvantage of fluorescein dyes, were not observed in the present experiments. The dye and the emitted fluorescence remained stable for over 10^5 laser pulses. The photo-stability is likely due to lower laser irradiances, the small absorption cross-section at 532 nm, and the short pulse length of the Nd:YAG laser.

In addition, performing a ratiometric technique in which the fluorescence intensity of FL27 is divided by the fluorescence intensity of Kiton Red 620 (KR) yielded a measurement that was more temperature-sensitive than a single dye tracer measurement or previously reported dual-tracer measurements. The spectral separation between the FL27 emission and the KR emission presents an amenable detection scheme that is plausible using commercially available filters and detectors. The proposed ratiometric technique was shown to be highly temperature-sensitive at high temperatures (~7% per °C at 50°C) when using 532-nm excitation of a Nd:YAG laser. The use of a Nd:YAG laser also provides the ability to acquire "instantaneous" images in transient or turbulent flowfields and temporally resolved measurements, although details such as adequate signal detection, signal-to-noise ratio, and spatial resolution specific to individual imaging applications must still be examined.

Acknowledgments This research is supported by the Office of Naval Research (ONR) through the Naval Research Laboratory core funding.

References

Arcoumanis C, McGuirk JJ, Palma JMLM (1990) On the use of fluorescent dyes for concentration measurements in water flows. Exp Fluids 10:177–180

Bowers PG, Porter G (1967) Triplet state quantum yields for some aromatic hydrocarbons and xanthene dyes in dilute solution. Proc R Soc Lond Ser A 299:348–353

Brackmann U (2000) Lambdachrome® laser dyes, 3rd edn. Lambda Physik GmbH, Göttingen

Bruchhausen M, Guillard F, Lemoine F (2005) Instantaneous measurement of two-dimensional temperature distributions by means of two-color planar laser induced fluorescence (PLIF). Exp Fluids 38:123–131

Coolen MCJ, Kieft RN, Rindt CCM, van Steehoven AA (1999) Application of 2-D temperature measurements in water using a Nd:YAG laser. Exp Fluids 27:420–426

Coppeta J, Rogers C (1998) Dual emission laser induced fluorescence for direct planar scalar behavior measurements. Exp Fluids 25:1–15

Crimaldi JP (1997) The effect of photobleaching and velocity fluctuations on single-point LIF measurements. Exp Fluids 23:325–330

Dahm WJA, Southerland KB, Buch KA (1991) Direct, high resolution, four-dimensional measurements of the fine scale structure of Sc >> 1 molecular mixing in turbulent flows. Phys Fluids A 3:1115–1127

Hidrovo CH, Hart DP (2000) Dual emission laser induced fluorescence technique (DELIF) for oil film thickness and temperature measurement. In: Proceedings of ASME FEDSM'00, ASME 2000 fluids eng. div. summer meeting, 2000, Boston, MA

Hidrovo CH, Hart, DP (2001) 2D Thickness and temperature mapping of fluids by means of a two dye laser induced fluorescence ratiometric scheme. In: Proceedings of PSFVIP-3, 2001, Maui, HI

Hishida K, Sakakibara J (2000) Combined planar laser-induced fluorescence-particle image velocimetry technique for velocity and temperature fields. Exp Fluids 29:S129–S140

Isak SJ, Eyring EM (1992) Fluorescence quantum yield of cresyl violet in methanol and water as a function of concentration. J Phys Chem 96:1738–1742

Karasso PS, Mungal MG (1997) PLIF measurements in aqueous flows using the Nd:YAG laser. Exp Fluids 23:382–387

Kim HJ, Kihm KD, Allen JS (2003) Examination of ratiometric laser induced fluorescence thermometry for microscale spatial measurement resolution. Int J Heat Mass Trans 46:3967–3974

Koochesfahani MM, Dimotakis PE (1985) Laser induced fluorescence measurements of mixed fluid concentration in a liquid plane shear layer. AIAA J 23:1700–1707

Koochesfahani MM, Dimotakis PE (1986) Mixing and chemical reactions in a turbulent liquid mixing layer. J Fluid Mech 170:83–112

Larson LG, Crimaldi JP (2006) The effect of photobleaching on PLIF. Exp Fluids 41(5):803–812

Lavieille P, Lemoine F, Lavergne G, Virepinte JF, Lebouché M (2000) Temperature measurements on droplets in monodisperse stream using laser-induce fluorescence. Exp Fluids 29:429–437

Lavieille P, Lemoine F, Lavergne G, Lebouché M (2001) Evaporating and combusting droplet temperature measurements using two-color laser-induced fluorescence. Exp Fluids 31:41–55

Lemoine F, Antoine Y, Wolff M, Lebouché M (1999) Simultaneous temperature and 2D velocity measurements in a turbulent heated jet using combined laser-induced fluorescence and LDA. Exp Fluids 26:315–323

Lide DR (ed) (1990) CRC handbook of chemistry and physics, 70th edn. CRC Press, Boca Raton

Lindqvist L (1960) A flash photolysis study of fluorescein. Arkiv Kemi 16:79–138

Lopéz Arbeloa I, Rohatgi-Mukherjee KK (1986) Solvent effects on photophysics of the molecular forms of rhodamine B. Internal conversion mechanism. Chem Phys Lett 129:607–614

Lopéz Arbeloa I, Lopéz Arbeloa T, Tapie Estévez MJ (1991) Photophysics of rhodamins. Molecular structure and solvent effects. J Phys Chem 95:2203–2208

Rehab J, Antonia RA, Djenidi L, Mi J (2000) Characteristic of fluorescein dye and temperature fluctuations in a turbulent near wake. Exp Fluids 28:462–470

Saeki S, Hart DP (2001) Investigation on YAG (532) laser dyes for oil film thickness and temperature measurement. In: Proceedings of the third pacific symposium of flow visualization and image processing (Paper index number F3096), March 18–21, 2001, Maui, Hawaii

Sakakibara J, Adrian R (1999) Whole field measurements of temperature in water using two-color laser induced fluorescence. Exp Fluids 26:7–15

Sakakibara J, Adrian R (2004) Measurement of temperature field of a Rayleigh-Bénard convection using two-color laser-induced fluorescence. Exp Fluids 37:331–340

Sakakibara J, Hishida K, Madeda M (1993) Measurements of thermally stratified pipe flow using image processing techniques. Exp Fluids 16:82–96

Saylor JR (1995) Photobleaching of disodium fluorescein in water. Exp Fluids 18:445–447

Seuntiëns HJ, Kieft RN, Rindt CCM, van Steehoven AA (2001) 2-D temperature measurements in the wake of a heated cylinder using LIF. Exp Fluids 31:588–595

Snavely BB (1969) Flashlamp-excited organic dye lasers. Proc IEEE 57:1390

Walker DA (1987) A fluorescence technique for measurements of concentration in mixing liquids. J Phys E Sci Instrum 20:217–224

Wang GR, Fieldler HE (2000) On high spatial resolution scalar measurement with LIF. Part 1: photobleaching and thermal blooming. Exp Fluids 29:257–264