

OREGON STATE UNIVERSITY

# Calibration of Laser Induced Fluorescence Thermometry Using 2'7' Dichlorofluorescein and Sulforhodamine B

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PH 403 Senior Thesis

With Dr. Vinod Narayanan

Jaryd Ulbricht

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## Abstract

An apparatus and method for calibrating planar laser induced fluorescence using a two-dye, two-color method was developed. A laser sheet was expanded from a 473 nm laser to excite dye solutions into higher energy states then fluoresced through spontaneous emission. The two fluorescent dyes selected for research and development were the primarily green emitting 2'7' dichlorofluorescein and the primarily red emitting sulforhodamine B. Planar laser induced fluorescence is used in thermal-fluids experiments as an accurate, non-invasive method for developing flow thermographs used to characterize the performance of experimental devices and methods. Two-color, two-dye methods are used because of their high accuracy, and potentially higher sensitivity relative to other thermography techniques because the temperature can be expressed as a function of only the fluorescence ratio of the two dyes and is independent of local laser intensity, which may vary considerably with time and space during an experiment. Calibration curves and relationships were developed that accurately correlated the local temperature of the solution to the intensity ratio of the dyes. Using a concentration of 2'7' Dichlorofluorescein of  $4.624 \pm 0.294 \times 10^{-6} M$  and a concentration of Sulforhodamine B of  $8.105 \pm 0.294 \times 10^{-6} M$  predicted temperature measurements had an uncertainty of 2.09 °C.

## Introduction

### Laser Induced Fluorescence

Laser-induced fluorescence (LIF) has provided an accurate and especially useful tool in the analysis of fluid flows and thermodynamics. Conventional methods of measuring temperature within a liquid by use of thermocouples and other intrusive measuring devices have proven unreliable as the physical presence of the temperature measuring device inhibits fluid flow. LIF has provided a non-intrusive method of temperature measurement that allows the characteristics of the flow to be unaffected as well as being able to define the temperature at any local point in a planar sheet of the liquid. In order to utilize an LIF system, temperature/intensity curves must be determined in order to calibrate the measuring device.

In this paper, the process of calibrating an LIF system will be explained in detail including the determination of optimum dye concentration ratios, construction of a calibration apparatus, and testing of such an apparatus.

There has been no small amount of research on LIF thermometry, but little has been done to document the calibration process. Proper concentrations of dye are critical to the success of any LIF process as well as the design of a proper calibration system. It is essential then to record this procedure for future use.

The irradiated intensity of the fluorescent dyes can be modeled by the following equation (Guilbault, 1990):

$$I = I_0 \beta_c \Phi (1 - e^{-\epsilon b C}) \quad 1$$

where  $I_0$  is the intensity of the laser,  $\beta_0$  is the collection efficiency,  $\Phi$  is the quantum efficiency,  $\epsilon$  is the molar absorptivity,  $b$  is the absorption path length and  $C$  is the concentration of the dye. If the argument of the exponent is small (e.g.  $\epsilon b C \ll 1$ ) then

$$e^x = \sum_{n=0}^{\infty} \frac{x^n}{n!} = 1 + x + x^2 + \dots$$

$$e^{-\epsilon b C} \approx 1 - \epsilon b C$$

$$\text{and } I \approx I_0 \beta_c \Phi \epsilon b C \quad 2$$

And the intensity can be seen to be linear in regards to all the explicit parameters.

### Two Dye Two Color Method

In order to accurately measure the temperature of the dye solution the fluorescent intensity must be normalized to the intensity of the laser light. This is a difficult and tedious task, but if two dyes are used and their intensities are measured simultaneously then the relative signals will both depend on the same optical path length and incident laser beam intensity. Therefore normalizing the dye fluorescence to the laser is no longer necessary if two dyes, Dye A and Dye B, are prepared in a single sample.

$$I_A = I_0 \beta_{Ac} \Phi_A \epsilon_A b C_A \quad 3$$

$$I_B = I_0 \beta_{Bc} \Phi_B \epsilon_B b C_B \quad 4$$

$I_A$  and  $I_B$  are the intensities of each dye. When dividing both intensity measurements by each other

$$\frac{I_A}{I_B} = \frac{\beta_{cA} \Phi_A \epsilon_A C_A}{\beta_{cB} \Phi_B \epsilon_B C_B} \quad 5$$

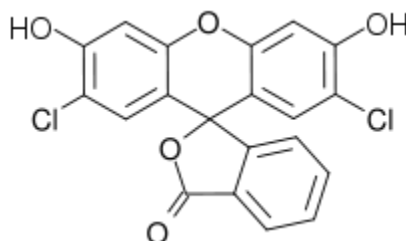
The incident laser sheet intensity and absorption path length,  $I_0$  and  $b$  respectively, drop out and their relative intensities will be constant even if the lasers intensity is not. Measuring the signal from the two dyes also allows us to make measurements deep into the sample and not have to account for the absorption (and therefore decreased absolute intensity) of the laser light.

### Experimental Dyes

2'7' Dichlorofluorescein (FL27) and Sulforhodamine B (SRhB) are the dyes being explored in this paper. Much previous work in LIF has used two dyes, one whose quantum efficiency varies linearly with temperature and one that remains constant. In contrast, by using two dyes whose temperature dependence is inversely related to each other (the quantum efficiency of FL27 increases linearly with temperature while the quantum efficiency of SRhB decreases with temperature) changes in temperature can be more accurately measured, decreasing the uncertainty in the results.

### 2'7' Dichlorofluorescein ( $C_{20}H_{10}Cl_2O_5$ )

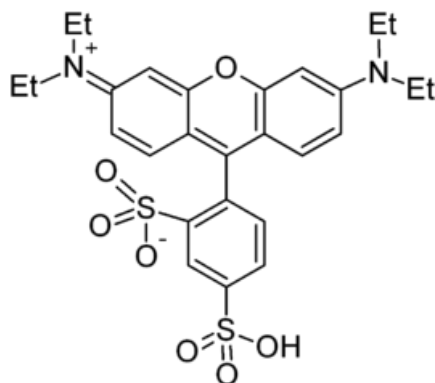
The excitation peak of FL27 is located at 500 nm (Figure 11: SRhB and FL27 Absorption Spectrum) and the emission peak is at 522 nm (Figure 1). FL27 is a derivative of Fluorescein, replacing the 2' and 7' positions with Chlorine.



The fluorescent intensity of FL27 is nearly independent of the pH of the solution (Figure 9) and has a molar mass of 401.2 g/mol. (National Institute of Advanced Industrial Science and Technology, 2012).

### Sulforhodamine B ( $C_{27}H_{30}N_2O_7S_2$ )

The excitation peak of SRhB (also known as Kiton Red) is located at 558 nm (Figure 11: SRhB and FL27 Absorption Spectrum) and the emission peak is at 579 nm (Figure 1). It is also commonly used as a biological tracer since it stains organic and other materials. Care should be taken with the material containing the dye solution, typical plastics will leach the SRhB, changing the concentration.



The fluorescent intensity of SRhB is independent of the pH of the solution (Figure 9) and has a molar mass of 558.666 g/mol. (National Institute of Advanced Industrial Science and Technology, 2012)

### Temperature Dependence of the Dyes

The quantum efficiency,  $\Phi$ , of the dyes is dependent upon the temperature of the dye solution, specifically the intensity of FL27 increases linearly with temperature while the intensity of SRhB

decreases linearly with temperature at about  $-2.6\% \text{ K}^{-1}$  (V K Natrajan, 2008). By manipulating the relationship between the intensities of each dye and modeling the quantum efficiency as

$$\Phi_A(T) = aT + b$$

$$\Phi_B(T) = d - cT$$

$T$  being the temperature while  $a$ ,  $b$ ,  $c$  and  $d$  are constants. A relationship between temperature and relative intensity can be found by substituting the quantum efficiency into the equation

$$\frac{I_A}{I_B} = \left( \frac{\beta_{cA}\epsilon_A C_A}{\beta_{cB}\epsilon_B C_B} \right) \frac{aT + b}{d - cT} = I_{AB} \quad 6$$

$I_{AB}$  represents the relative fluorescent intensity measurement of the dyes. The term in parenthesis is invariant with temperature and can therefore be treated as an arbitrary constant for calibration purposes and absorbed into the quantum efficiency.

$$I_{AB} = (\text{constant}) \frac{aT + b}{d - cT} = \frac{\alpha T + \beta}{\delta - \gamma T} \quad 7$$

Solving for temperature ( $T$ ) yields

$$T = \frac{\delta}{\gamma} \left( \frac{I_{AB} - \frac{\beta}{\delta}}{\frac{\alpha}{\gamma} + I_{AB}} \right) \quad 8$$

The constants are arbitrary and are picked to fit the temperature/intensity curve, so we do away with the fractions and retain only three constants.

$$T = A \left( \frac{I_{AB} - B}{\Gamma + I_{AB}} \right) \quad 9$$

Figure 1 shows the emission spectra of both FL27 and SRhB as a function of wavelength using a fluorometer to measure the fluorescent intensity. The large spike at 532 nm is residual laser light captured from a Nd:YAG 532 nm laser used to excite the dyes (see Methods: Determining Dye Concentration). A band-pass filter (dashed purple line) excludes the emissions of SRhB and a high-pass filter (dashed orange line) excludes emissions from FL27. The dyes emission peaks are at two distinct wavelengths, hence this two dye method is also commonly referred to as ‘two color’ LIF.



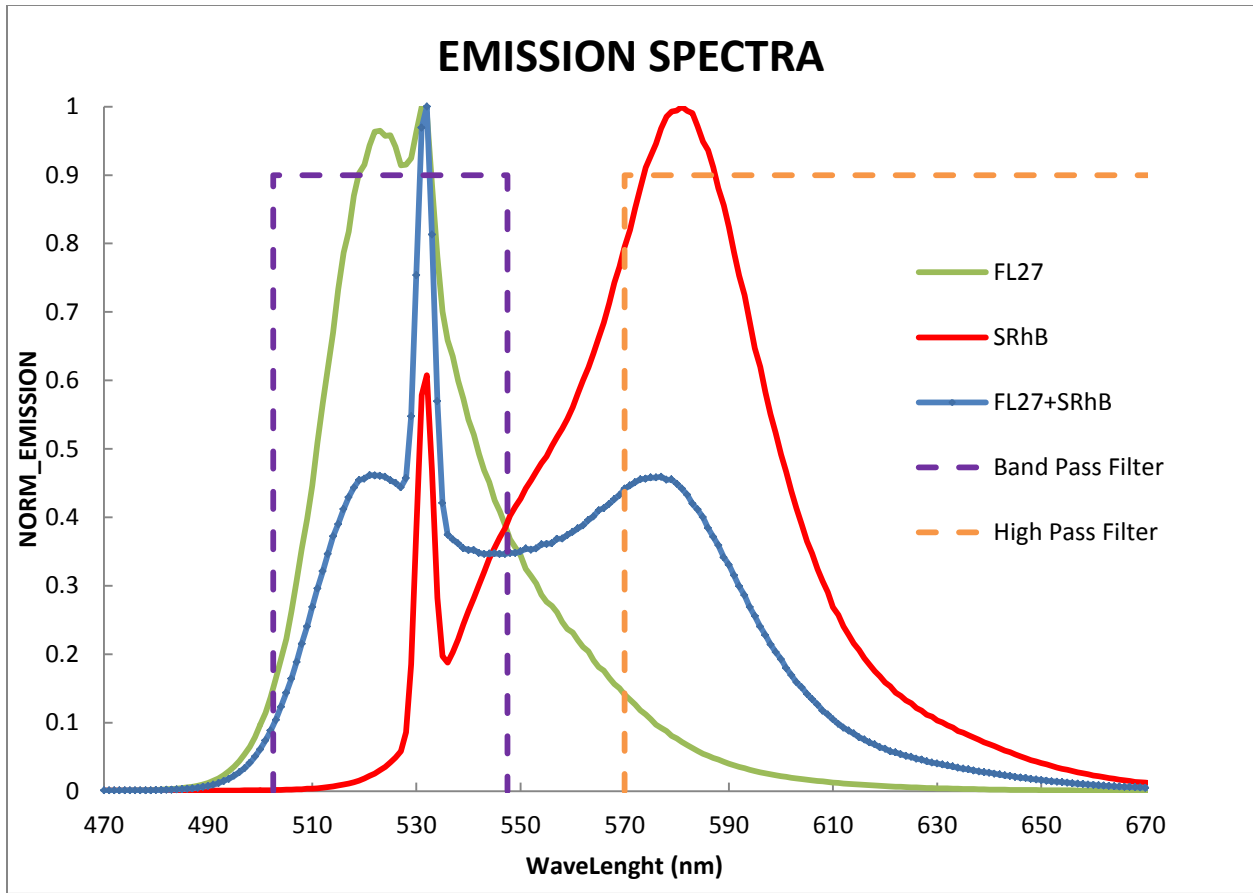


Figure 1: Emission spectra of FL27 and SRhB.

## Methods

### Experimental Set Up

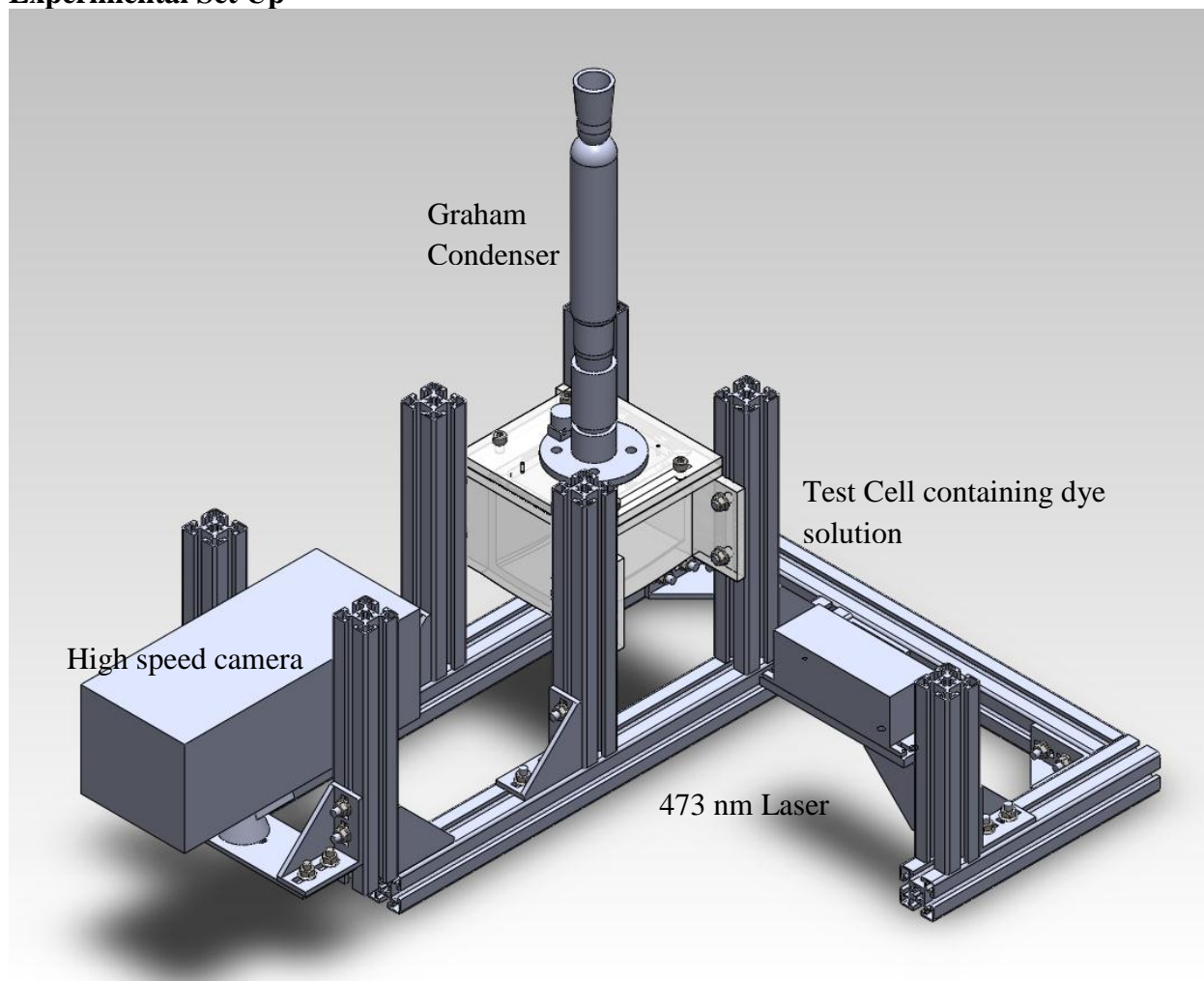


Figure 2: Solid model of experimental design

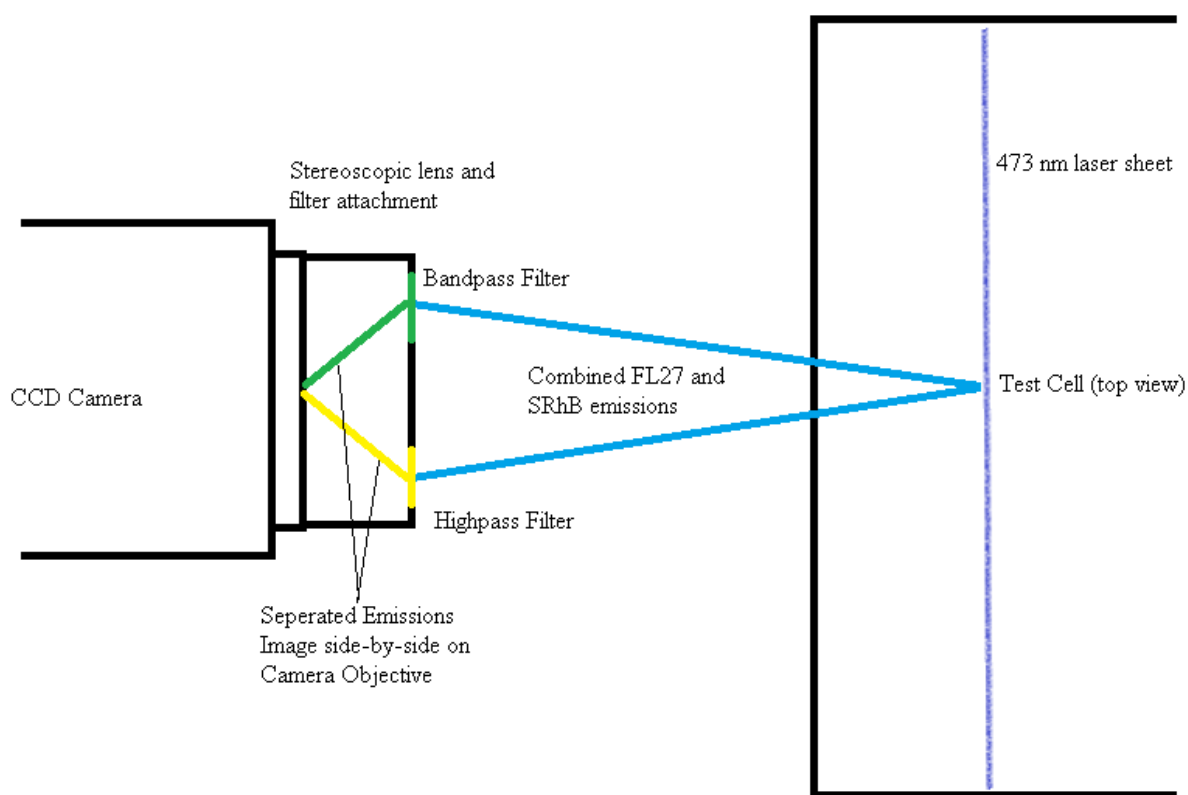
A cylindrical lens was used to create a planar laser sheet on one side of the test cell from a Sintec Optronics DPSS laser mounted on aluminum railing. The CCD high-speed camera was mounted with a macro stereoscopic lens used for viewing two near-identical images with a single camera. A high-pass filter (561 nm) and a band-pass filter (525-545 nm) were attached to the front of the stereoscopic lens each covering one aperture.



**Figure 3: Stereoscopic Lens without filter attachment**



**Figure 4: Stereoscopic Lens and camera with filter attachment. On the right is the bandpass filter and on the left is the high pass filter**



**Figure 5: Lens and filter array diagram**

The lens was focused on the laser sheet in the test cell as shown in Figure 5. The stereoscopic lens places two near identical images on the camera objective, each image being separately filtered for each dye. In Figure 7 and Figure 8 the filtering of the images can clearly be seen at 24 °C and 65 °C respectively. Each half of the image is actually identical, just filtered for different wavelengths. This method of resolving the desired wavelengths absolves us of the need of needing two cameras to image both dye emissions simultaneously.

To account for the shift in images from the stereoscopic lens, a transformation script was written into an analysis program which calibrated the images by comparing them to a checkered

background before testing began (Figure 6). The bandpass filter passed fewer wavelengths than the highpass filter, as a result the intensity was decreased on that half of the image and had to be accounted for when determining dye concentrations.

The camera viewed the cell oriented orthogonal to the laser path and normal to the surface of the cell to reduce imaging refracted laser light. The entire apparatus (laser, camera and test cell) was mounted on a single aluminum frame for ease of use.

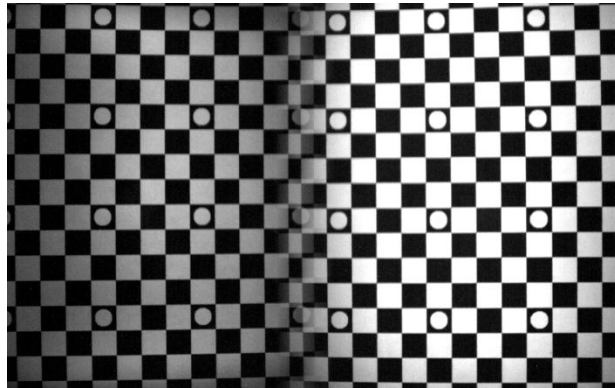


Figure 6: Registration Calibration Image

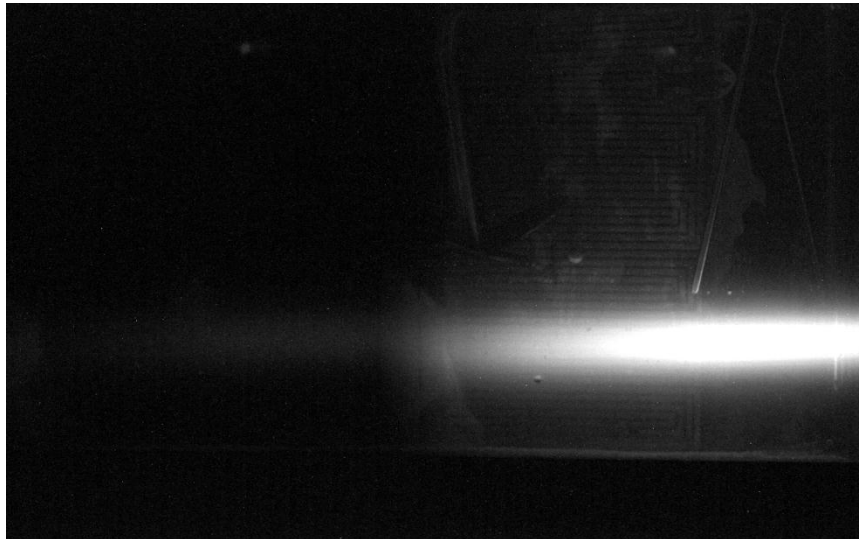


Figure 7: Camera image of both dyes (FL27 left and SRhB right) at 24 ° C

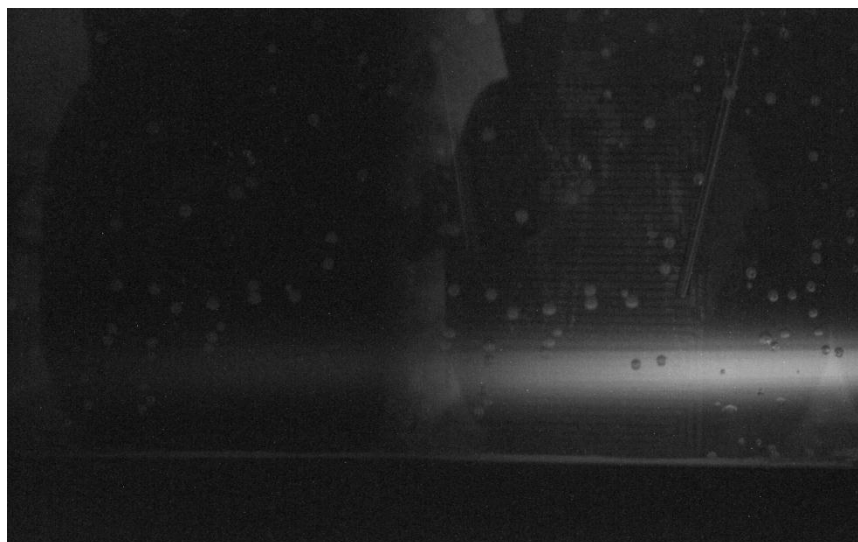


Figure 8: Camera image of both dyes (FL27 left and SRhB right) at 65° C

Four K-type thermocouples were placed within the cell to record temperature data which was correlated to the relative signal of the dyes using a LabView program.

To heat the dyes a resistive heat pad was glued to the inside of the cell using VRC epoxy (VRC epoxy was used because it doesn't react with the dyes). After testing we found that part of the heat pad wasn't completely submerged in the solution and caused part of the tank wall to melt when heating. An attempt to insulate the heat pad from the test cell wall was made by coating it in VRC epoxy, but it still restricted the temperature range we could achieve without destroying the test cell.

### Determining Dye Concentrations

FL27 is somewhat pH dependent, and its fluorescence appeared to vary with the concentration of SRhB as well (see Figure 9: pH dependency of dyes. The intensity spike at 532 nm is from a Nd:YAG laser captured by the camera).

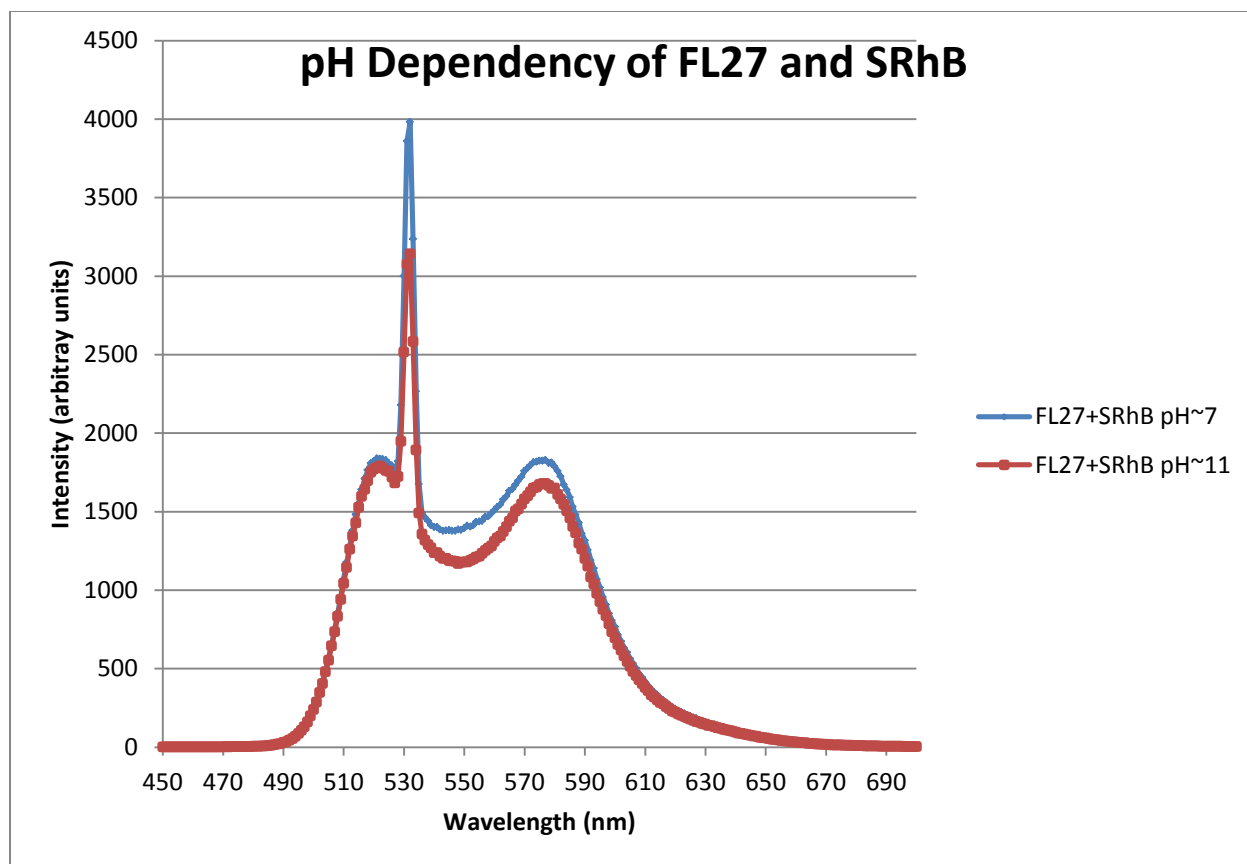


Figure 9: pH dependency of dyes. The intensity spike at 532 nm is from a Nd:YAG laser captured by the camera

It was therefore very important to eliminate any variation in concentration during the testing procedures. One of the causes of concentration discrepancy was evaporation of water from the solution as it was heated. To reduce this effect a Graham condenser was affixed to the top of the test cell. This returned the water to the sample quickly enough that the volume of the solution remained stable during the testing and also opened the cell to atmospheric pressure reducing any pressure dependent variables in the intensity.

The dye solution was heated from 24° C to 90 ° C and allowed to reach a steady state at several temperatures while the camera captured images. To ensure even heating a stir rod attached to a motor was suspended above the test cell and ran during the entire test. Nine points were plotted during the test of the relative intensities of dyes and then graphed against the temperature of the test cell.

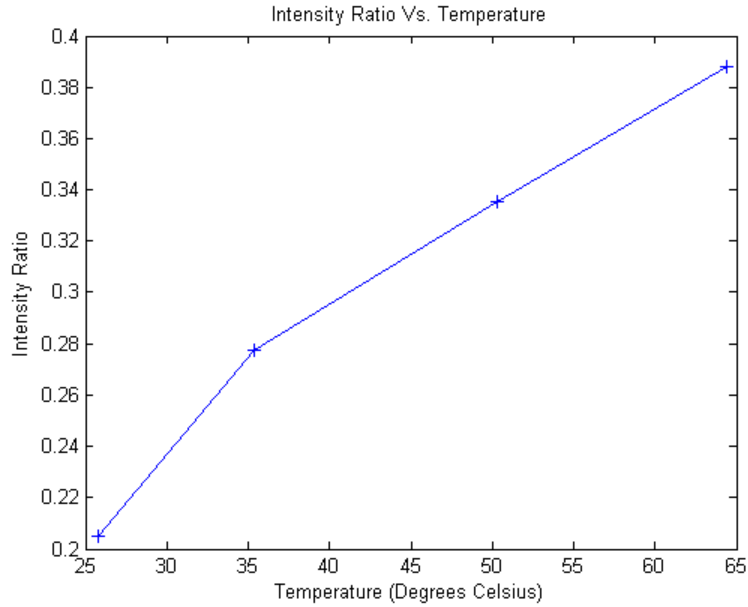


Figure 10: Early experimental data depicting the intensity ratio as a function of temperature

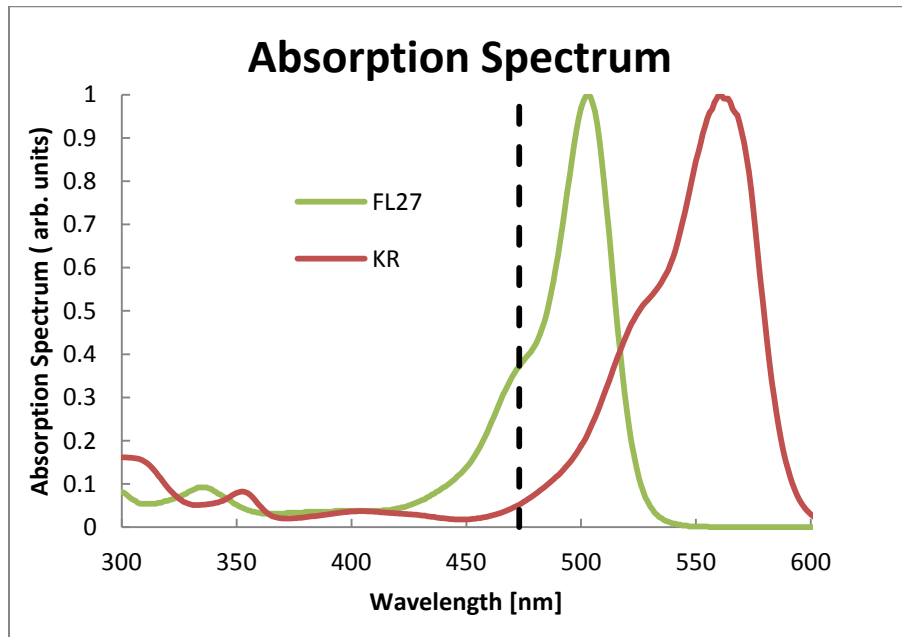


Figure 11: SRhB and FL27 Absorption Spectrum

Figure 11: SRhB and FL27 Absorption Spectrum shows the absorption spectrum of the dyes with the dashed vertical line representing the 473 nm excitation wavelength of the DPSS laser. This excitation wavelength was used because, as shown in Figure 1, using a Nd:YAG 532 nm laser resulted in a spike in the data near the FL27 emission peak. This background would have had to

been removed by the use of a notch filter in order to get accurate results. Rather, a 473 nm excitation wavelength was used, which was still within the absorption range of both FL27 and SRhB.

To determine the most efficient dye concentration a fluorometer was employed. Multiple samples were prepared using a high concentration of dye then diluting them down several orders of magnitude using deionized (DI) water. The two dyes were mixed and measured simultaneously since they appeared react with each other and exhibited unpredicted behavior. The fluorometer was able to both scan the emission spectrum of the dyes and provide relative intensity readings at specific wavelengths. The excitation wavelength was set to 473 nm with a 2.5 nm slit width using a xenon bulb. 4 mL cuvettes containing the dye solution were inserted into the fluorometer. For each sample 5 intensities were recorded and then averaged at  $585 \pm 2.5$  nm and also  $520 \pm 2.5$  nm in order to record data at both emission peaks. The fluorometer integrated for one second before displaying a measure of the intensity at each wavelength.

Since SRhB decreases intensity with temperature and FL27 increases both dyes must emit at nearly the same amplitude and at least a few orders of magnitude above background readings to help eliminate error in the measurements.

## Results

Preliminary testing showed that the intensity of FL27 depended very much on the concentration of SRhB. Much care was therefore taken in determining the final concentration of the dye solution.

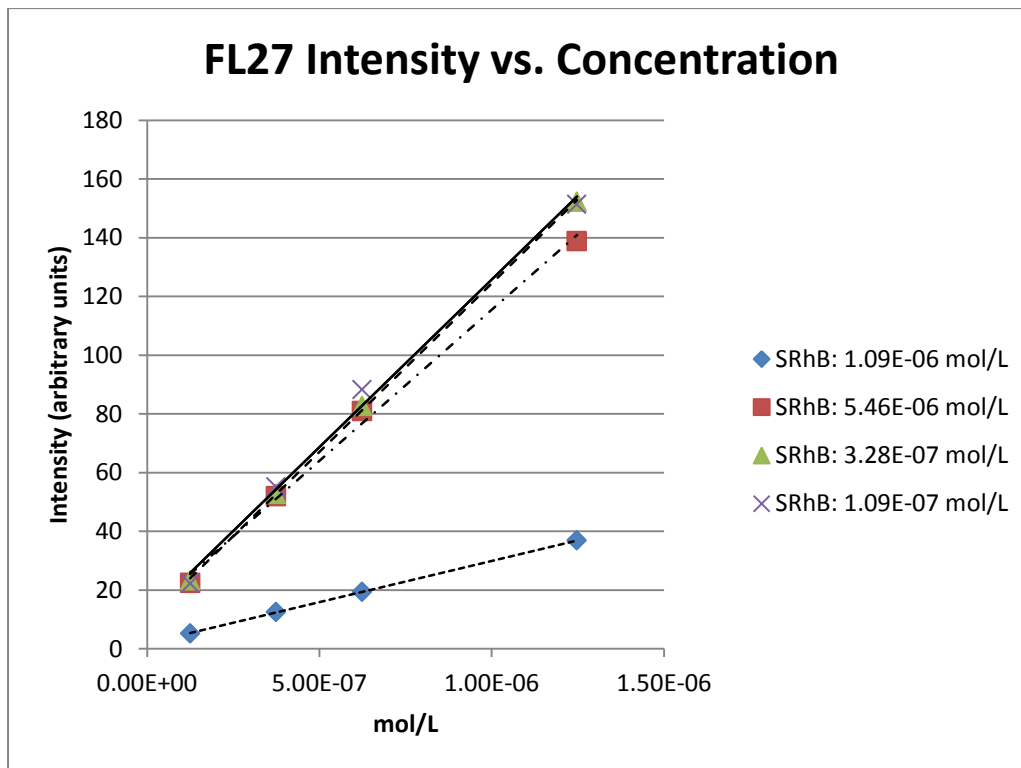


Figure 12: FL27 intensity dependence on SRhB concentration



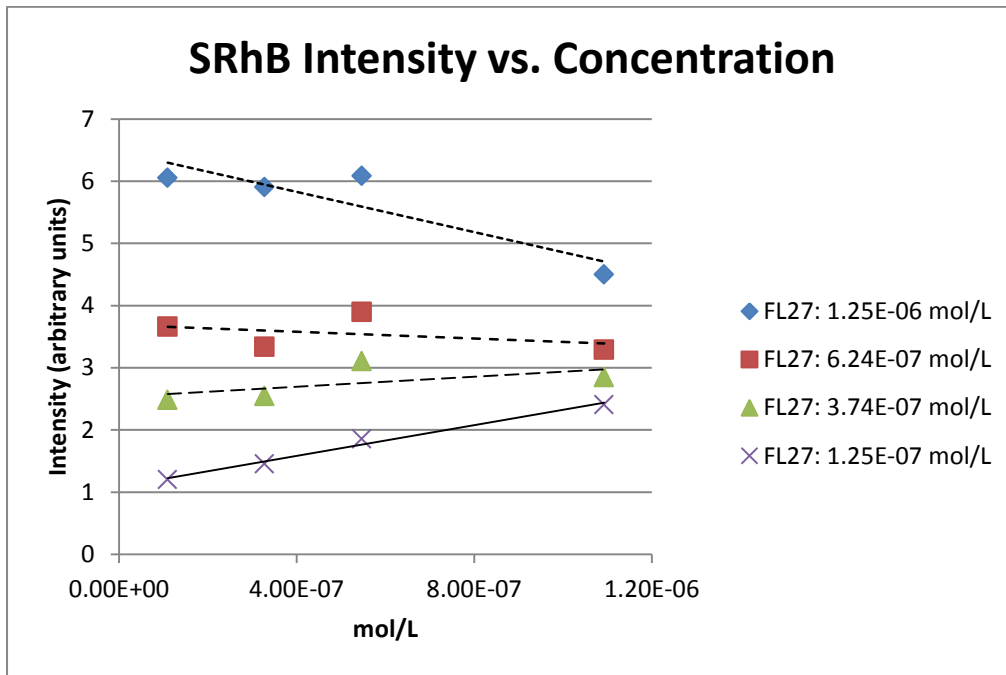
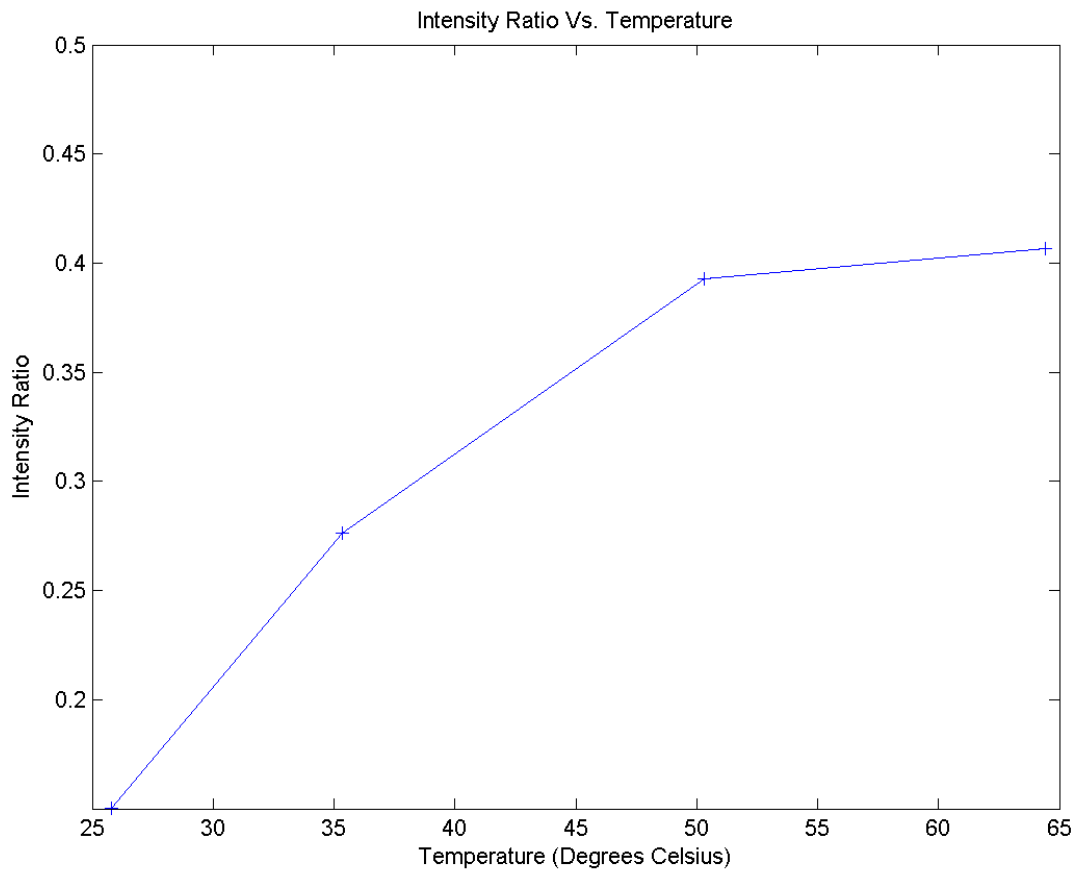


Figure 13: Intensity of SRhB showing low resolution

Early testing of SRhB fluorescent intensity as a function of concentration showed more dependence on concentration of FL27 than on the concentration of SRhB (Figure 13). Filters were not used in preliminary testing using the fluorometer.

Figure 14: Initial Calibration is the resulting calibration curve from the first calibration run performed. The concentration in this run was  $1.321 \pm 0.294 \times 10^{-6} M$  for FL27 and  $1.0507 \pm 0.0294 \times 10^{-5} M$  for SRhB. Since the signal was low from FL27, its concentration was increased to  $4.624 \pm 0.294 \times 10^{-6} M$  and the concentration of SRhB was decreased to  $8.105 \pm 0.294 \times 10^{-6} M$  for the second run. Figure 15: Final Calibration is the resulting calibration curve from the second calibration run. Though the concentration of FL27 was increased, the intensity on the left half of Figure 8 decreased from Figure 7. This could mean that the light being passed by the band-pass filter was fluorescence from SRhB at shorter wavelengths.

Though the calibration curves generated seem promising since they increase steadily with temperature, it is not conclusive whether or not the emission from FL27 is being measured effectively. The variability between the two runs suggests that work needs to be done with the laser and camera optics setup in order to increase repeatability. Also, the region of the image chosen by the user in which to perform the analysis has a significant impact on the resulting ratios. The use of additional image processing steps such as limiting the analysis points based on intensity and statistical criteria should be investigated.



**Figure 14: Initial Calibration**

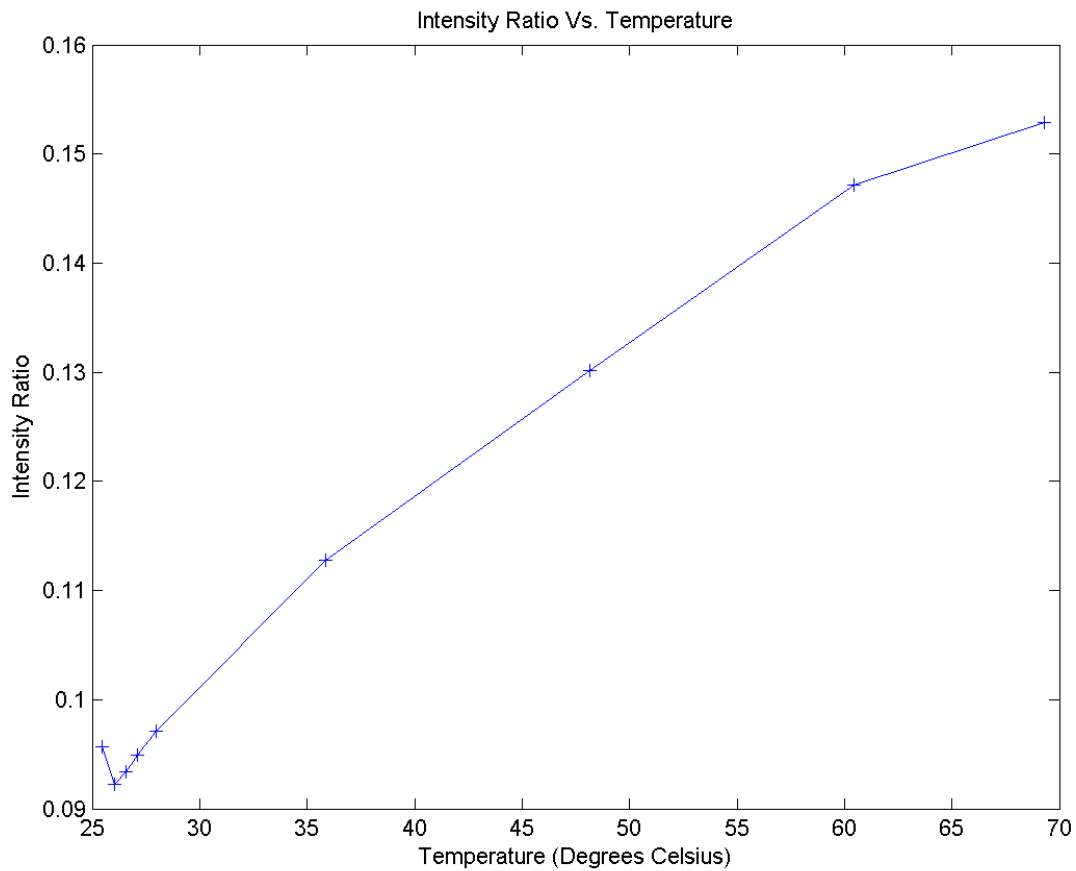


Figure 15: Final Calibration

Fitting the data to a linear regression we found the constants in equation **Error! Reference source not found.** to be

$$A = 3036.3640$$

$$B = 0.0603$$

$$C = 4.1136$$

Resulting the following model with stated uncertainty.

$$T = 9655.1724 \left( \frac{I_{AB} - 0.0579}{13.4483 + I_{AB}} \right) \pm 2.09 \text{ } ^\circ\text{C}$$

Experimental		Theoretical	
Intensity Ratio	Temperature	Temperature	$\Delta$ Temperature
0.097	25.3727	26.4982	1.12552
0.09358	25.9317	24.0515	-1.8802
0.09484	26.4907	24.9534	-1.5373
0.09628	26.9565	25.9834	-0.9731
0.09844	27.8882	27.5272	-0.361
0.11374	35.7143	38.417	2.70268
0.13084	48.1056	50.495	2.3894
0.1474	60.4037	62.0992	1.69548
0.15298	69.2547	65.989	-3.2656

Figure 16: Experimental results and deviation from theoretical model

## Analysis

### Dye Concentration Error

The process of creating dyes and the uncertainties of each of the measuring devices used must be known. Dye solutions are made by creating individual solutions of the dyes A and B beforehand then mixing them with an amount of deionized (DI) water. The concentration of dye A in solution 1 is

$$C_{a1} = \frac{m_a V_{a1}}{M_a V_a V_1} \quad 10$$

where  $m_a$  is the mass of dye A used in the individual solution,  $V_{a1}$  is the amount of individual solution used in the final solution,  $M_a$  is the molar mass of dye A,  $V_a$  is the amount of DI water used in the individual solution, and  $V_1$  is the total volume of solution 1. The error is given by

$$e_{C_{a1}} = C_{a1} \left[ \left( \left( \frac{1}{V_{a1}} - \frac{1}{V_1} \right) e_{V_{a1}} \right)^2 + \left( \frac{e_{V_{b1}}}{V_1} \right)^2 + \left( \frac{e_{V_a}}{V_a} \right)^2 + \left( \frac{e_{m_a}}{m_a} \right)^2 + \left( \frac{e_{V_0}}{V_1} \right)^2 \right]^{1/2} \quad 11$$

For a typical dye solution, the error is about

$$e_{C_{a1}} = 4 \times 10^{-6} M \left[ \left( \left( \frac{1}{20 \text{ mL}} - \frac{1}{1000 \text{ mL}} \right) 0.05 \text{ mL} \right)^2 + \left( \frac{0.05 \text{ mL}}{1000 \text{ mL}} \right)^2 + \left( \frac{0.05 \text{ mL}}{100 \text{ mL}} \right)^2 + \left( \frac{0.0005 \text{ g}}{0.008024 \text{ g}} \right)^2 + \left( \frac{0.6 \text{ mL}}{1000 \text{ mL}} \right)^2 \right]^{1/2} = 2.49 \times 10^{-7} M$$

Since this result is so low, additional errors such as evaporation of water during mixing or dye powered clinging to the weighing paper are unlikely to increase the error above the target value.

**Determining Calibration Curve**

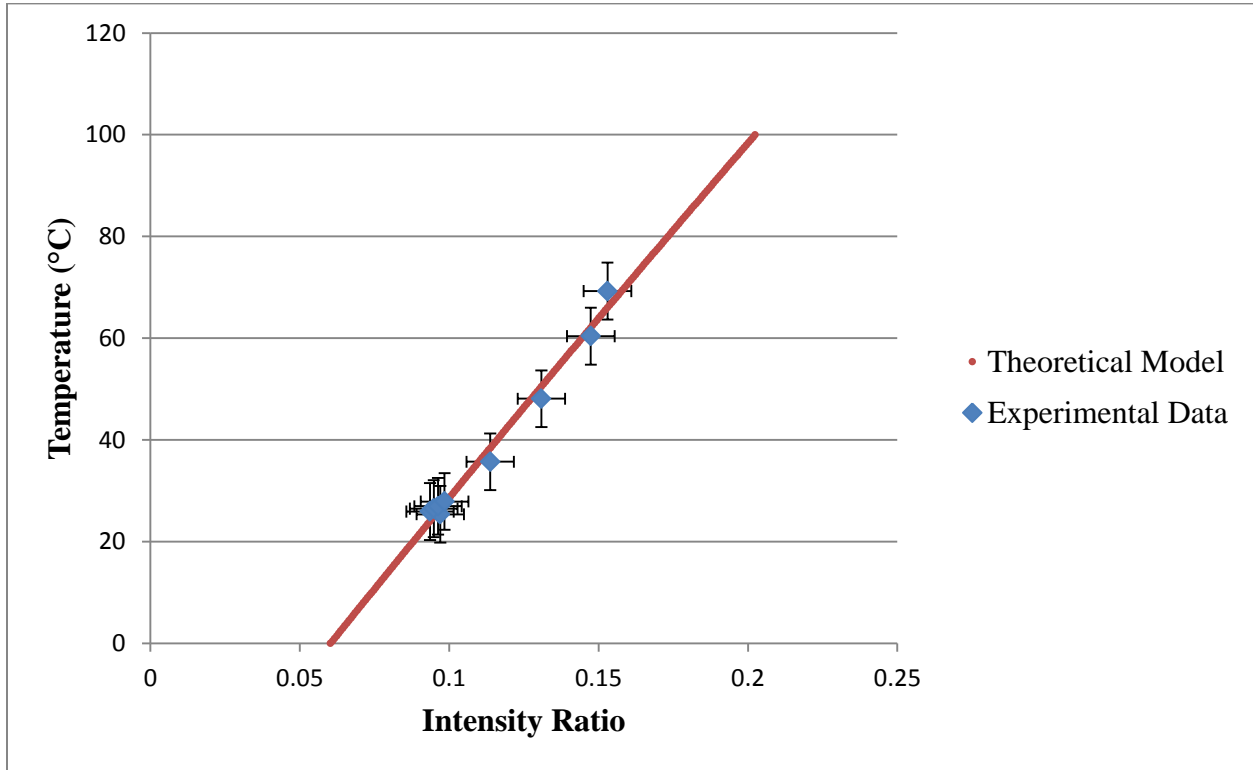


Figure 17: Experimental Results

Figure 17 shows a good correlation between the experimental data and the theoretical model developed for the particular dye concentrations. The model was developed by fitting the results using linear regression and then determining the constants A, B and Γ such that a linear regression fit of the theoretical model over the region of interest was identical to the fit of the experimental data.

After analyzing the data, using the temperature deviation from Figure 16: Experimental results and deviation from theoretical model, the uncertainty in our results was ±2.09 °C.

**Conclusions**

A linear regression model was used to determine experimentally the behavior of the relative dye intensities as a function of the temperature of the dye solution, yielding the model below

$$T = 9655.1724 \left( \frac{I_{AB} - 0.0579}{13.4483 + I_{AB}} \right) \pm 2.09 \text{ } ^\circ\text{C} \tag{12}$$

To determine the temperature of the dye solution at any point in the fluid all that is needed is the relative intensities of the dyes. Plugging the fluorescent signal of the dyes into equation 9 then gives a quick and easy evaluation of the temperature. The concentrations of the dyes used were

$4.624 \pm 0.294 \times 10^{-6} M$  for 2'7' dichlorofluorescein and  $8.105 \pm 0.294 \times 10^{-6} M$  for sulforhodamine B.

Discrepancies in the data were likely due to a low registered signal from FL27 as a result of the smaller bandgap of the bandpass filter compared to the highpass filter. The decreasing slope of the data at higher temperatures seems to correlate to a decrease in the signal from SRhB captured through the bandpass filter, simulating a decreased response of the FL27. Suggested future work would be the further experimentation with different dye concentrations to yield a better signal.

Suggestions for future work would be to study the behavior of the dyes in more detail. Specifically, study the effects on the calibration curve from increasing the concentration of FL27. As well a new method of heating should be researched that will allow the calibration apparatus to reach high temperatures. A more efficient optical array that could expand the laser into a larger planar sheet might also be beneficial.

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