Research Square

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Spectral Characteristics of Water-Soluble Rhodamine Derivatives for Laser- Induced Fluorescence

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Abstract

We present a comprehensive fluorescence characterization of seven water-soluble rhodamine derivatives for applications in laser-induced fluorescence techniques (LIF). Absorption and emission spectra for these dyes are presented over the visible spectrum of wavelengths (400 to 700 nm). Their fluorescence properties were also investigated as a function of temperature for LIF thermometry applications. Rhodamine 110 depicted the least fluorescence emission sensitivity to temperature at -0.11%/°C, while rhodamine B depicted the most with a -1.55%/°C. We found that the absorption spectra of these molecules are independent of temperature, supporting the notion that the temperature sensitivity of their emission only comes from changes in quantum yield with temperature. Conversely, these rhodamine fluorophores showed no change in emission intensities with pH variations and are, therefore, not suitable tracers for pH measurements. Similarly, fluorescent lifetime, which is also a property sensitive to local environmental changes in temperature, pH, and ion concentration, measurements were conducted for these fluorophores. It was found that Rhodamine B and Kiton Red 620 have shorter fluorescence timescales compared to those of the other five rhodamine dyes, making them least suitable for applications where temporal changes in emission are monitored. Lastly, we conducted experiments to assess the physicochemical absorption characteristics of these dyes' molecules into PDMS, the most common material for microfluidic devices. Rhodamine B showed the highest diffusion into PDMS substrates as compared to the other derivative dyes.

1. Introduction

Many physical and engineering applications, such as the assessment of heat transfer characteristics in fluid flows and visualization of species mixing, often involve the use of fluorescent dyes since these tracers can probe the physical parameters associated with these phenomena [1–6]. Laser-induced fluorescence (LIF), a versatile tool for the visualization and quantification of changes in temperature [1, 7], pH [8, 9], and local concentrations [10–12], is well equipped for these purposes. Fluorescence in LIF is an inelastic radiative process involving the absorption and subsequent emission of a photon following quantum energy state interactions. By measuring fluorescence emissions from dye molecules affected by environmental conditions, LIF can be used to construct a local spatiotemporal map of these quantities.

Selection of the most suitable fluorophores is an important step in any LIF system optimization and characterization process [13, 14]. However, this selection process is usually costly and time-consuming since multiple tests are involved that require considerable amounts of materials and equipment in order to accurately determine the properties of the chosen fluorophores. The comprehensive study presented here, which examines the absorption and emission characteristics of a family of dyes according to environmental conditions, can be used to assess the accuracy and suitability of the different fluorophores for specific LIF applications.

Rhodamine derivatives are the most popular fluorophores suitable for LIF since they have several excellent photochemical properties compared to other fluorescent molecules [15–20]. Some desirable properties of rhodamine derivatives are that 1) they absorb a broad band of wavelengths covering the 400 nm – 650 nm range (i.e., they allow flexibility in terms of excitation sources) [21], 2) their quantum yields are relatively high compared to other fluorophores (~ 30% higher), such as fluorescein [21–24], and 3) they are highly resistant to photobleaching (a phenomenon by which a dye loses its ability to fluoresce under extended photoexcitation) [25–27]. Generally, they are photochemically stable in dry powder form in dark environments, but they have also shown great promise as indicators of physical properties when they are dissolved in hydrocarbon-based solvents such as ethanol and methanol [28]. Hence, their absorption and emission spectra in hydrocarbon solutions are extensively available in the literature [28–31]. While these hydrocarbon-based solvents provide high solubility of the rhodamine dyes, many chemical and engineering applications require different solvents, such as water, glycerol, deuterium oxide, and dimethyl sulfoxide.

Water can undoubtedly be considered the cleanest solvent available, greatly contributing to “green chemistry” [32]. In addition, water is essential in organic chemistry for synthesis and reactions [33]. Advantages of using water as a solvent include fast reaction rates, non-toxicity, and low cost [34]. It is also nonflammable and environmentally friendly and can prevent the dissolution of organic gases, a major consideration in air-sensitive catalysis [32]. For aqueous-based LIF applications, water-soluble fluorophores that can probe changes in system properties are required. Water-soluble rhodamine derivatives are a great choice for this purpose due to the aforementioned advantages. While some studies have used one or two specific water-soluble rhodamine dyes for temperature or concentration measurements [12, 17, 35], there is no study that extensively compares the absorption and emission spectra of the many available water-soluble rhodamine derivatives and systematically quantifies their pH and temperature dependent characteristics.

In this paper, we present spectral characteristics of seven water-soluble rhodamine derivatives whose emissions cover most of the visible wavelengths $\lambda =$ 420 nm – 650 nm. Their absorption and fluorescence spectra were obtained at room temperature when dissolved in deionized (DI) water. The temperature sensitivities of these rhodamine derivatives were also obtained in the 26–55°C range. Our most important conclusion was that the absorption spectra of rhodamine derivatives are independent of temperature. This implies that the temperature sensitivity of the fluorescence emission presumably comes from changes in their quantum yields with temperature rather than their absorption spectra. Similarly, their fluorescence emissions were characterized as a function of pH. For a demonstration of pH-dependent emissions of rhodamine derivatives, a CO_2 dissolution-driven pH change was induced in DI water for fluorescein and rhodamine B solutions in a PMMA-based microchannel. It was found that rhodamine B displays no changes in intensity with pH variations, unlike fluorescein. In addition, their fluorescent lifetimes, a key parameter in LIF applications for cancer research [36], human skin disease [37], and stem cell research [38], were characterized. Finally, using fluorescence microscopy, the absorption and diffusion of these rhodamine derivatives in PDMS, a representative material used in aqueous LIF applications, were also measured.

This is a unique, comprehensive study and report that summarizes absorption and emission spectra for water-soluble rhodamine dyes, their behavior with respect to temperature and pH, fluorescence lifetimes, and diffusivity into PDMS. Although some of this information is available in certain papers dealing with single or dual LIF systems, the library of rhodamine derivatives and their behavior is quite limited and scattered. The results from this work serve as a comprehensive database for researchers needing to use water-soluble rhodamine derivatives as tracers in LIF-based techniques and applications.

2. Experimental Methods

2.1 Instruments

The absorption spectra at room temperature were measured by a CARY 5000UV-VIS NIR spectrophotometer (Varian, USA) with an excitation slit of 1nm. All measurements carried out with this instrument were calibrated by the absorption spectrum of a base solvent. Emission spectra at room temperature were recorded on a FluoroLog®-3 spectrofluorometer (HORIBA Scientific, USA) with a 450 W xenon CW lamp as the illumination source. A clear, four-sided, 1 cm cuvette was used with a 420 nm excitation source alongside a 1 nm emission slit for spectra collection. The fluorescence was measured at 90° to the incident excitation beam from 450nm to 700nm. The fluorescence intensities were calibrated against the fluorescence of the base solvent (water in this study) and the detector response. Temperature-dependent absorption and emission spectra were measured by a SpectraMax microplate reader (Molecular Devices, USA). Using fluorophore solutions at a 10 μM concentration, eight fluorophores, including Fl and seven rhodamine derivatives, were transferred to a 96-well plate. The microplate reader provides automatic calibration for background noise and temperature-controlled measurements. Measurements were made for each temperature after the microplate reader was auto-calibrated against a built-in thermocouple.

2.2. Reagents and materials

DI water was prepared by removing contaminants from tap water with a filtration system. All rhodamine derivatives were purchased from Exciton (Exciton Inc., USA) and used as received. Both sodium phosphate monobasic and dibasic were purchased from Sigma. Citric monohydrate and sodium hydroxide were obtained from Fisher Scientific.

2.3. Preparation of pH solution

To produce a pH 8.5 fluorophore solution, 0.1M phosphate sodium salt was dissolved in DI water. An appropriate amount of fluorophore solution at 5 μmol/L from the 1 mmol/L stock was then added to the pH buffer. To produce a pH 6.5 solution, sodium hydroxide was used to titrate the pH of the DI water. To produce a pH 4.5 solution, 0.1 M citric acid and sodium hydroxide were used to titrate the pH values similarly as described above.

2.4. Measurement of fluorescence lifetime

For fluorescent lifetime measurements, changes in time-dependent photon counts were recorded by the FluoroLog®-3 spectrofluorometer under ambient conditions. A four-sided clear cuvette containing the fluorophore solution was placed into a holder, and a diode laser at 421 nm excited the sample with a bandwidth of 1nm. A photomultiplier measured the number of photons from the sample at 20 kHz with an emission bandwidth of 1 nm. By exciting the sample with the laser at a repetition rate of 10 MHz, time-resolved measurements of fluorescence lifetime were made. Specifically, time-correlated single photon counting was recorded based on the detection of the arrival times of individual photons to the photomultiplier immediately after the 300ps laser pulse excitation commences. This excitation-emission process was repeated many times by building up a histogram of the number of counts versus time.

2.5. Diffusion of dye molecules into PDMS

Polydimethylsiloxane (PDMS) is a permeable material to gas and organic molecules. It is well known that RhB, an organic fluorescent dye, significantly diffuses into cured PDMS. In this study, diffusion and absorption of seven rhodamine derivatives, including RhB, into PDMS was visualized and quantified by fluorescence microscopy. After curing PDMS microchannels, they were placed on a microscope stage so that the field of view was the same for each chip. A 10 μM aqueous dye solution was then injected by a syringe pump at 10 μL/min. A reference image at $t = 0$ s was taken immediately after the solution was injected, and a second image was taken 60 minutes later. Fluorescence emissions from each dye solution throughout the microchannel section and adjacent wall sections were recorded by a 10x objective lens onto a CCD camera.

2.6. CO_2 dissolution-induced pH change

DI water with the 10 μM concentration of fluorescein (FL 116) and rhodamine B (RH115), respectively, were used to test their pH sensitivity. The pH of aqueous solutions was changed by continuous CO_2 dissolution as the CO_2-water reaction produces two hydrogen ions from the subsequent dissociation of dissolved CO_2 (*aq*). To test the pH sensitivity of different dyes, a polymethylmethacrylate (PMMA) microchannel (100 μm (W)×100 μm (H) ×30 mm (L)), possessing excellent optical access to imaging of fluorescence, was prepared (Fig. 1). Each test solution was injected into the PMMA microchannel with a syringe pump with a 90 μL volume. Once the channel was filled halfway through with the test solution, the outlet port was closed while the inlet was immediately connected to a CO_2 gas line (the gas valve had not been turned on at this point). It is important that the time step to switch the water line to the gas line must be as short as possible because CO_2 dissolution started before the imaging began. The solution-filled microchannel was exposed to 0.5 psig CO_2 gas over time when the gas pressure was maintained constant during the test. The time-dependent emission intensities were recorded by a camera for image processing.

3. Results and Discussion

3.1. Absorption and emission spectra

Figure 2a shows an image depicting Fl (yellow-green) and seven rhodamine derivatives: (rhodamine 110 (R110), rhodamine 123 (R123), rhodamine 590 (R590), rhodamine 610 (R610 or RhB), kiton red 620 (K620), rhodamine 640 (R640), and sulforhodamine 640 (S640)) under ambient light conditions at 21°C. Fl, a popular fluoropore for use in LIF, was chosen as a baseline for comparison purposes. The color of the rhodamine derivatives ranges from yellow-green (R123) to dark pink (S640), indicating that their emissions range from ~ 500 nm to ~ 600nm. Although R123, R110, and R6G are discernable in the figure, RhB, K620, R640, and S640 are difficult to distinguish by color due to the similarity of their emission spectra.

Figure 2b and 2c show absorption and emission spectra for Fl and seven rhodamine derivatives at a 10 μM concentration, while their maximum absorption and emission values are tabulated in Table 1. For the rhodamine derivatives, the seven dyes combined cover a wavelength range from 420nm to 650nm. It is noteworthy that four of these fluorophores, R610, K620, R640, and S640, have a second absorption peak at a shorter wavelength, making their absorption range broader than other similar dyes. This second absorption peak characteristic also results in a second peak of fluorescence at longer wavelengths (Fig. 2c). These broadening effects are beneficial, especially when these fluorophores are employed in LIF techniques because 1) there is more flexibility in the selection of the excitation sources (e.g., Ar + laser at 488, 514 nm, Nd:YAG laser at 532 nm), and 2) various combinations of fluorophores are possible in dual-tracer applications by minimizing crosstalk between two wavelength bands [1, 7].

Table 1
Absorption and emission maximum for Fl and seven rhodamine dyes

Dye	Fl	R110	R123	R590	RhB	K620	R640	S640
Wavelength at absorption maximum (nm)	490	496	500	526	554	565	586	586
Wavelength at emission maximum (nm)	515	521	534	554	580	588	609	610

3.2. Temperature and pH dependence

LIF provides a practical, non-invasive approach to measuring liquid temperatures that has been extensively used in different applications [1, 7, 35]. The temperature-dependent characteristics on the quantum yield of some dye molecules allow for thermometry by monitoring the fluorescence intensity changes with temperature. To accurately measure temperatures using LIF, it is essential to carefully choose the appropriate temperature-sensitive tracers. Figure 3 shows the temperature-dependent fluorescence spectra for Fl and the seven rhodamine derivatives picked in this study. All spectra were obtained in a temperature-controlled spectrometer (SpectraMax, USA) at 27, 35, 45 and 55°C. It is well known that Fl is a good temperature tracer with a strong temperature-sensitive emission [1], as seen in Fig. 3 (a). The temperature sensitivity of the rhodamine dyes varies depending on the specific fluorophore. The emission of Rhodamine 110 shows no temperature dependence, suggesting that this dye serves as a good reference dye when no temperature sensitivity is desired. On the other hand, all the

other derivatives show temperature-dependent emissions, with a uniform decrease in intensity across the emission spectrum but without distortion or shift on its wavelength profile.

Table 2 shows quantitative results of changes in both the emission maximum and the total amount of emission as temperatures change from 26°C to 55°C. There are very slight differences in the exact percentage change between the peak fluorescence intensity and the sum of the total emission intensities as a function of temperature, mostly due to experimental error, but the overall characteristics of change are essentially the same. Fl shows about 31% change of emission, resulting in a temperature sensitivity of -1.08%/°C. Among the rhodamine derivatives, the dyes that show the largest changes in their emissions are R610 and K620, showing a temperature sensitivity of -1.55 and – 1.53%/°C, respectively. These sensitivities are in agreement with the literature [1, 7]. If a dual fluorescence LIF approach is to be used, a tracer pair combination of a temperature-sensitive dye and a temperature-insensitive dye is typically required. By taking the ratio of two emissions, the source of errors due to fluctuation of excitation intensity can be minimized. As long as both dyes are excitable by the same illumination source, it is apparent that the combination of R610-R110 shows the highest temperature sensitivity and, therefore, provides the most accurate temperature measuring system.

Table 2
Changes in emissions of Fl and rhodamine derivatives with temperatures and their average temperature sensitivity in the range of 27–55°C.

	Fl	**R110**	**R123**	**R590**	**R610**	**K620**	**R640**	**S640**
% change of emission maximum*	33.1	0.4	10.6	14.5	46.5	46.4	20.3	18.3
% change in total emission (based on area)*	31.5	3.3	9.2	14.3	45.0	44.6	18.8	16.1
Temperature Sensitivity (%/°C)**	-1.08	-0.11	-0.31	-0.49	-1.55	-1.53	-0.64	-0.55
*Temperature changes from 27 to 55°C								
**When curve-fitted to a linear								

Temperature-dependent fluorescence occurs because either the quantum yield or the fluorophore's absorption spectrum is sensitive to temperature changes. Fl is a well-known fluorophore with different temperature sensitivities when excited at different wavelengths due to its temperature-sensitive absorption characteristics [1]. For example, Fl has a temperature sensitivity of -0.16%/°C when excited at 488 nm, while it has 2.43%/°C at 514nm excitation. To investigate the effect of temperature on the fluorescence of the rhodamine derivatives, their absorption spectra were measured at temperatures of 27, 35, 45, and 55°C. Figure 4 shows representative absorption spectra for R610 and S640 dyes. It is apparent from Fig. 4 that the absorption spectra of all derivatives are insensitive to temperature. These results support the notion that the temperature sensitivity of their emissions is unaffected by the

excitation wavelength. Rather, the emissions change with temperature, apparently due to variations in quantum yield with temperature.

Another important aspect to consider when using organic fluorescent molecules as tracers for measurements of physical properties is their sensitivity to solution pH. Changes in pH cause variations in emission intensities due to the pH-dependent absorptivity of the dye molecules. Figure 5 shows the emission spectra for the representative rhodamine dyes R123 and S640 that exhibit emission characteristics independent of solution pH over the 4.5, 6.5, and 8.5 values. Although rhodamine dyes are a good choice as tracers for temperature measurements in environments with variable pH conditions, other organic fluorophores, such as Fl, are better choices for pH measurements.

As one of the representative demonstrations for pH measurements with these dyes, we measured pH changes in DI water with fluorescein and rhodamine B upon continuous CO_2 injection into test solutions. Because of cascading hydrogen ion production from CO_2-water reactions, the carbonated solution by CO_2 becomes acidic, lowering the solution pH. Figures 6(a) and (b) show changes in emission intensities of both fluorescein and rhodamine B dyes at 6 different time steps along the microchannel when the solution was pressurized by a CO_2 gas. In the case of fluorescein dye, the emission changed from the meniscus (not shown in the figure) of the CO_2-water interface. Since a CO_2 gas continuously dissolves into the test solution (from the righthand side of the figure), the solution pH becomes acidic. Figure 6(a) shows an intensity decrease of fluorescein's emission over time due to its pH sensitivity. On the other hand, emission intensities of rhodamine B remained constant over time upon CO_2 injection, as expected, because of the pH-insensitivity of its emission intensity.

3.3. Fluorescence lifetime

Fluorescence lifetime (τ), defined as the average time that a molecule resides in the excited state before returning to its ground state, is an intrinsic property of the fluorophore. Due to the high sensitivity of the fluorescence lifetime to environmental conditions, measurements of the fluorophore's lifetime can be used to evaluate changes in pH [39–42], temperature [43–45], and ion concentration[46, 47]. For example, Sanders et al.[39] used the ratio of fluorescence lifetime measurements in two different time windows to map pH values over Chinese hamster ovary cells with a carboxy SNAFL-1 whose fluorescence lifetime is pH-dependent. Similarly, Nakabayashi et al. [42] used fluorescence lifetime imaging microscopy (FLIM) to image the intracellular pH of HeLa cells by using the pH-dependent ionic equilibrium of the chromophore from enhanced green fluorescent proteins. In order to measure intercellular temperature distributions in a living COS7 cell, Okabe et al. [44] employed the temperature-dependent fluorescent lifetime of a fluorescent polymeric thermometer that diffuses throughout the entire cell, combined with a time-correlated single photon counting system-based FLIM. In addition, Tremier et al. [48] utilized FLIM to determine fluorescence resonance energy transfer (FRET) in living cells as a powerful method for visualizing protein-protein interactions and biochemical reactions. They studied the impact of photobleaching on the measured fluorescent lifetime from fluorescent proteins such as CFP, YFP, and GFP in FRET applications. Lastly, Bopp et al. [49] used fluorescence lifetime

measurement to estimate the photobleaching time of one bacteriochlorophyll molecule by using a confocal fluorescence microscope with picosecond time resolution.

Fluorescence emission occurs when the excited fluorophore molecules return to their ground state releasing energy in the form of visible light during this process. This process and the corresponding fluorescence intensity follow an exponential decay over time that can be expressed as:

$I(t) = I_0 \exp(-t/\tau)$,

where I_0 is the maximum intensity of the fluorescent emission at the population inversion point. The fluorescence lifetime is defined as the time taken for this peak fluorescence intensity to decay to $1/e$ (37%) of its value. Although fluorescence derives from a stochastic excitation-relaxation process, its intensity initially increases as the number of molecules that populate the excited state increases until reaching its maximum intensity, I_o, at the onset of the population inversion point before gradually decaying.

In the current study, we measured the fluorescence lifetime of the seven rhodamine derivatives under the same environmental conditions, specifically room temperature (23°C), atmospheric pressure, and pH 6.5. Figure 7 shows the fluorescence lifetime measurements for Fl and the seven rhodamine dyes in DI water. It is apparent that there are two distinct groups of fluorescence lifetimes for the rhodamine dyes: one for RhB and K620, and another one for the rest of the rhodamine derivatives (Table 3). These results indicate that the emissions of RhB and K620 have shorter decay times (less than 1.74 ns) than the other rhodamine dyes (decay times of 4.53 ns and more). Due to the large range in fluorescence lifetimes (1.71 ns to 5.08 ns), in terms of absolute lifetime values, these rhodamine derivatives seem comparable to the fluorophores used in the aforementioned applications above. For example, the lifetime of carboxy SNAFL-1 is 1.1 ns, while those of GFP and YFP are 3.5 and 4.5 ns, respectively. However, to ensure that these rhodamine dyes are competitive against these other tracers for the purposes of measuring environmental pH and temperature changes, further work on the dependence of their fluorescent lifetimes as a function of these variables is needed.

Table 3
Fluorescence lifetime for Fl and seven rhodamine dyes

Dye	Fl	RhB	K620	R123	R110	R590	S640	R640
Fluorescence Lifetime (ns)	3.74	1.71	1.74	4.53	4.66	4.80	4.92	5.08

3.4. Diffusion into PDMS

PDMS-based chips are well suited to LIF techniques due to their transparency and ease of fabrication [50, 51]. However, they have been shown to have issues with absorption and diffusion of small molecules into their surfaces and bulk [52, 53]. However, to date, no study has compared the quantitative degree of permeability of rhodamine derivatives into PDMS microchannels. To test their permeability into the PDMS material, each of the seven rhodamine derivatives was mixed in solution with DI water at a

concentration of 10 μM and injected into a 100 μm-square PDMS microchannel. Two fluorescence images of the microchannel were taken at 0 and 60 minutes after the solution was injected at a flow rate of 10 μL/min. Figure 8 shows greyscale images of the fluorescence emission when RhB was injected into the chip at 0 min (Fig. 8a), depicting no initial absorption or diffusion of the dye solution into the PDMS and after 60 min (Fig. 8b). By continuing to expose the microchannel to the RhB solution, a large amount of RhB molecules start to permeate into the PDMS walls and subsequently penetrate into the PDMS bulk, producing a smoother fluorescence profile that gradually decays from the wall to the interior of the sample. After 60 min of injection, it is apparent that significant diffusion of RhB molecules into the PDMS material has occurred, as shown in Fig. 8b. To quantify and compare the degree of diffusion between the seven rhodamine molecules, the intensity profiles at t = 60 min were analyzed against the profile at t = 0 min as shown in Fig. 8c. As can be seen in the figure, the intensity profile of RhB is more pronounced towards the PDMS bulk material as compared to the others, suggesting that the diffusion of these molecules is more significant. Quantitatively, RhB penetrates into the PDMS material up to 30% of the microchannel width, while the others penetrate into the PDMS bulk by only 5% of its width.

4. Conclusion

Laser-induced fluorescence is a versatile tool that can non-intrusively measure several physical parameters such as temperature, pH, and concentration using organic fluorophores. In this technique, water is often used as a solvent due to its low cost and biocompatibility. In this study, we measured the absorption and fluorescence characteristics of seven water-soluble rhodamine dyes for their use in different LIF applications. The absorption and fluorescence spectra of these molecules were measured in 26–55°C range for temperature measurements application. We found that rhodamine B has the strongest temperature dependence with − 1.55%/°C. We also found that the temperature sensitivity of the emissions of all rhodamine derivatives comes from changes in quantum yield with temperatures rather than absorptivity. Conversely, the rhodamine fluorophores tested showed no change in emission intensities with pH variation, suggesting that these rhodamine derivatives are not appropriate options for pH measurements. Fluorescent lifetime measurements of the dyes showed that rhodamine B and kiton red 620 had a shorter timescale compared to the other five dyes suggesting that these latter ones might provide a better dynamic range in FLIM measurements. Furthermore, the lifetimes from the rhodamine family span the timescales of other common tracers making them a viable alternative for aqueous applications. Finally, it was found that rhodamine B shows the strongest propensity to diffusion into PDMS, suggesting that this dye should be avoided in applications employing this material, while no significant penetration of the other dyes to PDMS was observed after one hour, making them more appropriate for these purposes.

Declarations

Ethical Approval

Not applicable as the study does not include any use of animals and humans.

Consent to Publish

All authors mentioned in the manuscript have consented to submission and publication.

Competing Interests

The authors declare no competing interests.

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Author Contribution

A.R.: methodology, testing, data acquisition, data curation, writing-original draft preparation. M.M.: supervision, conceptualization, methodology, testing, data acquisition, data curation, writing-original draft preparation, reviewing, and editing. Y.K.: methodology, testing, data acquisition, data curation. C.H.: supervision, conceptualization, methodology, data curation, writing - reviewing, and editing.

Data Availability

No datasets were generated or analyzed during the current study.

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Figures

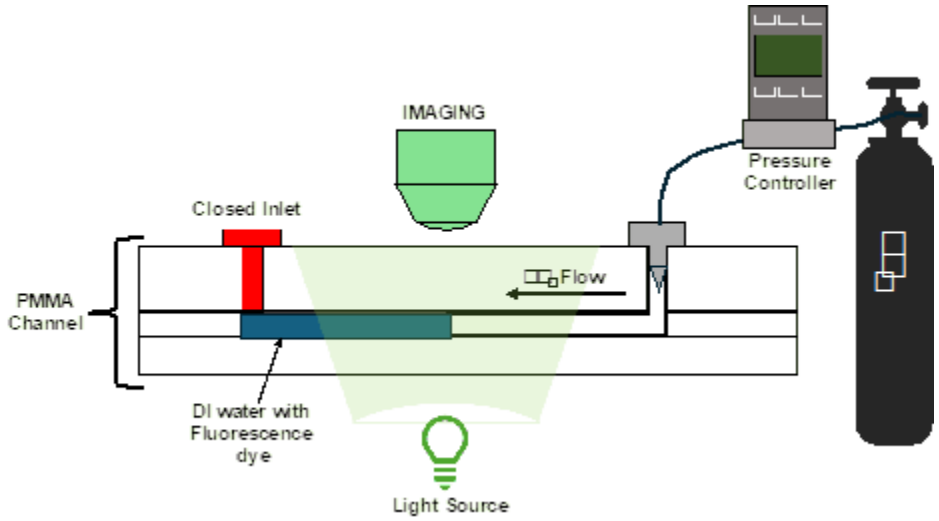


Figure 1

An illustrative experimental diagram for the CO_2 dissolution-induced pH changes of fluorescein and rhodamine B solutions in a PMMA microchannel.

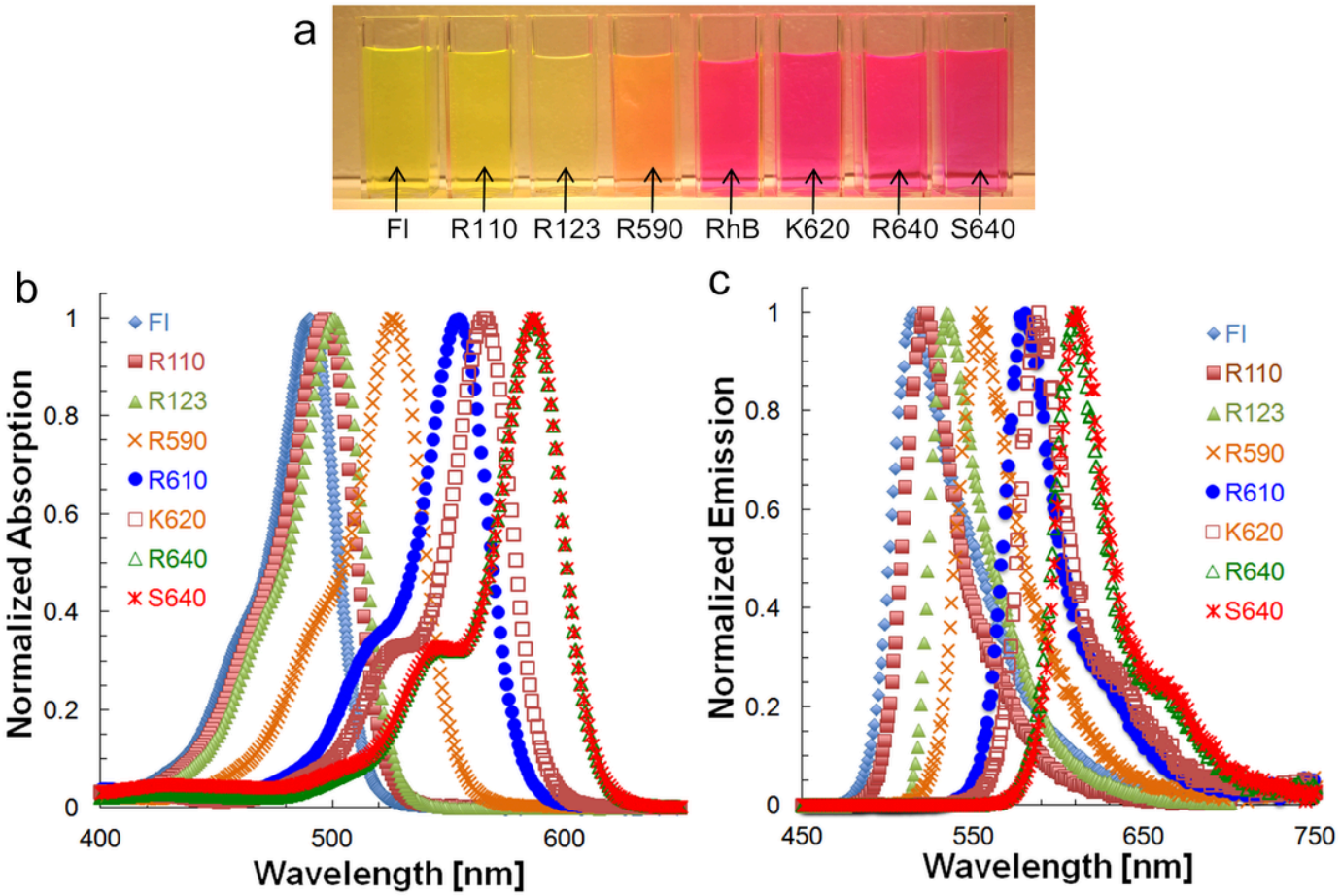


Figure 2

(a) A photograph, (b) absorption spectra, and (c) emission spectra for fluorescein and seven rhodamine derivatives.

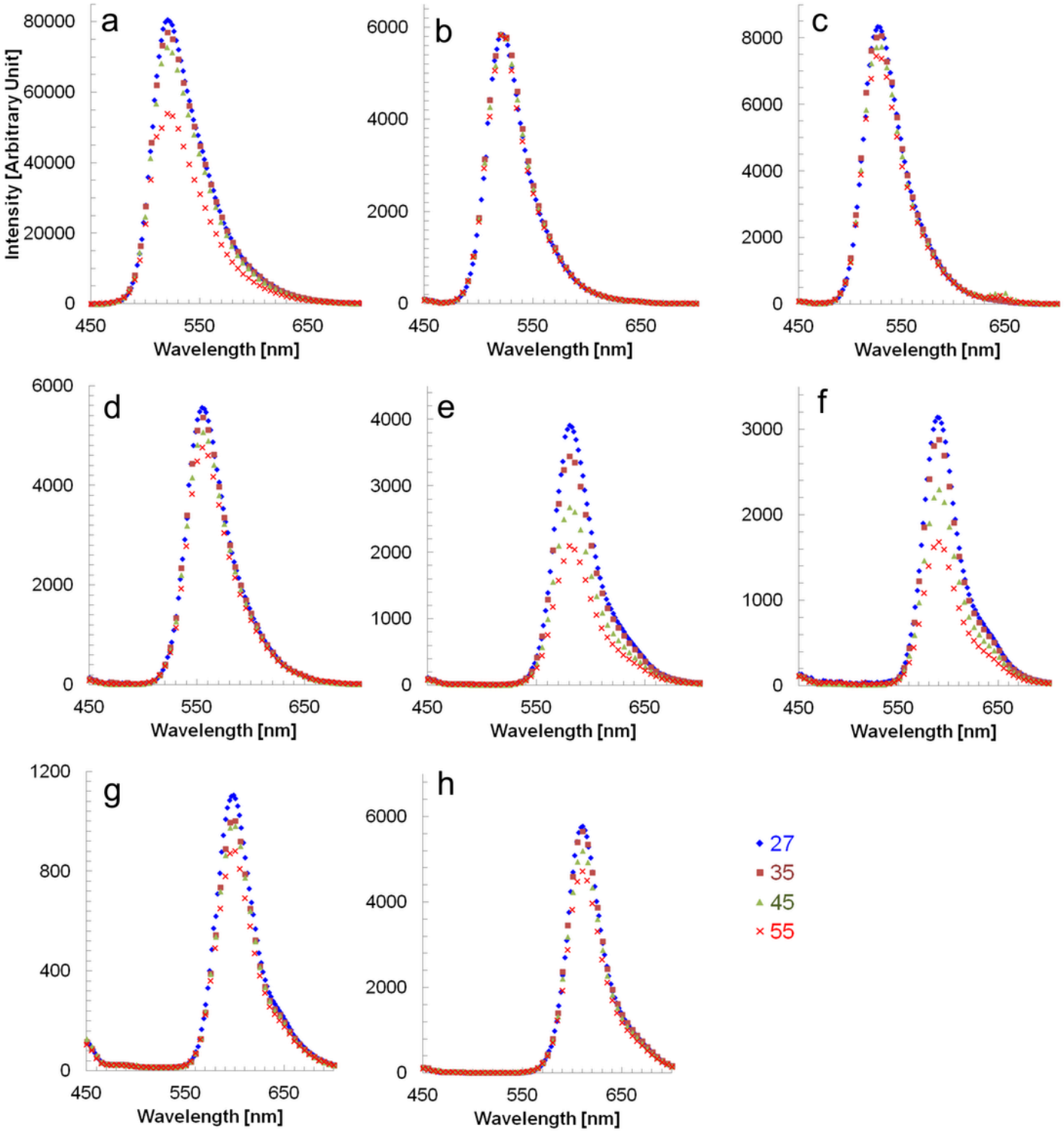


Figure 3

Emission spectra for fluorescein and rhodamine derivatives at different temperatures: (a) Fl, (b) R110, (c) R123, (d) R590, (e) R610, (f) K620, (g) R640, and (h) S640.

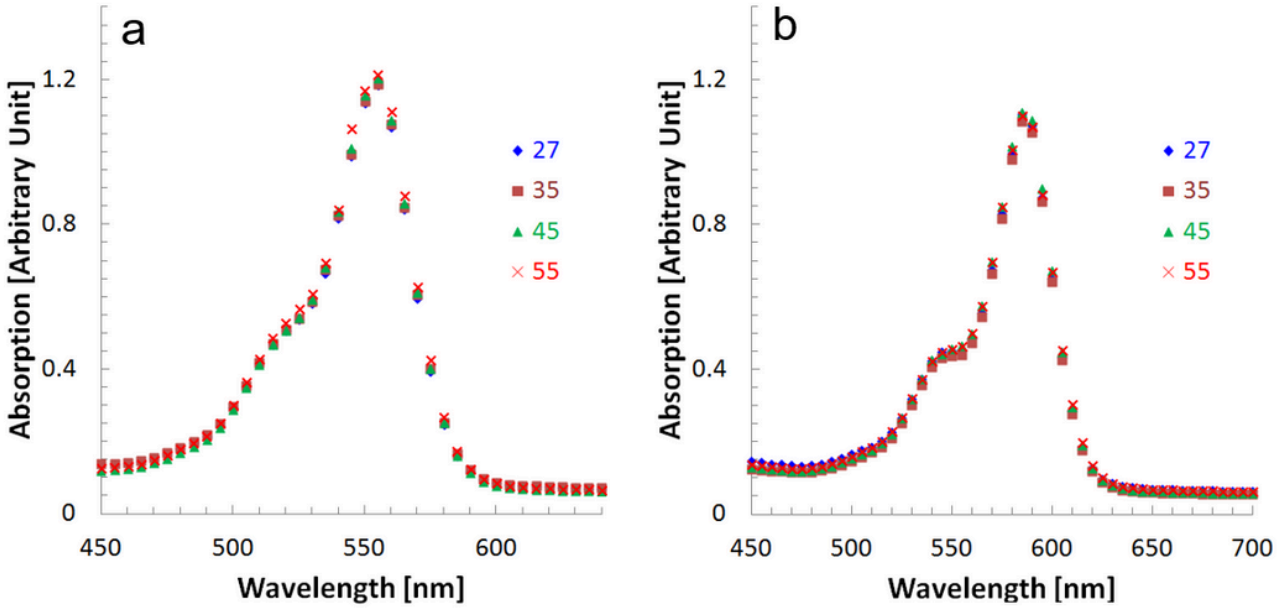


Figure 4

Absorption spectra of R610 and S640 at different temperatures.

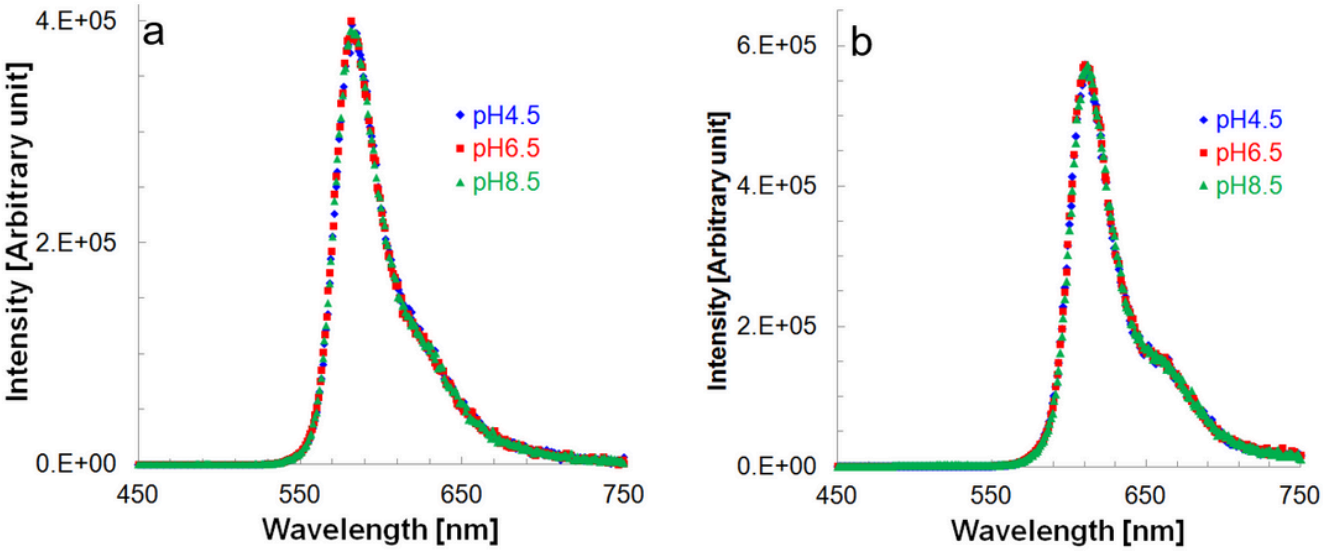


Figure 5

Emission spectra of R123 and S640 at different solution pH.

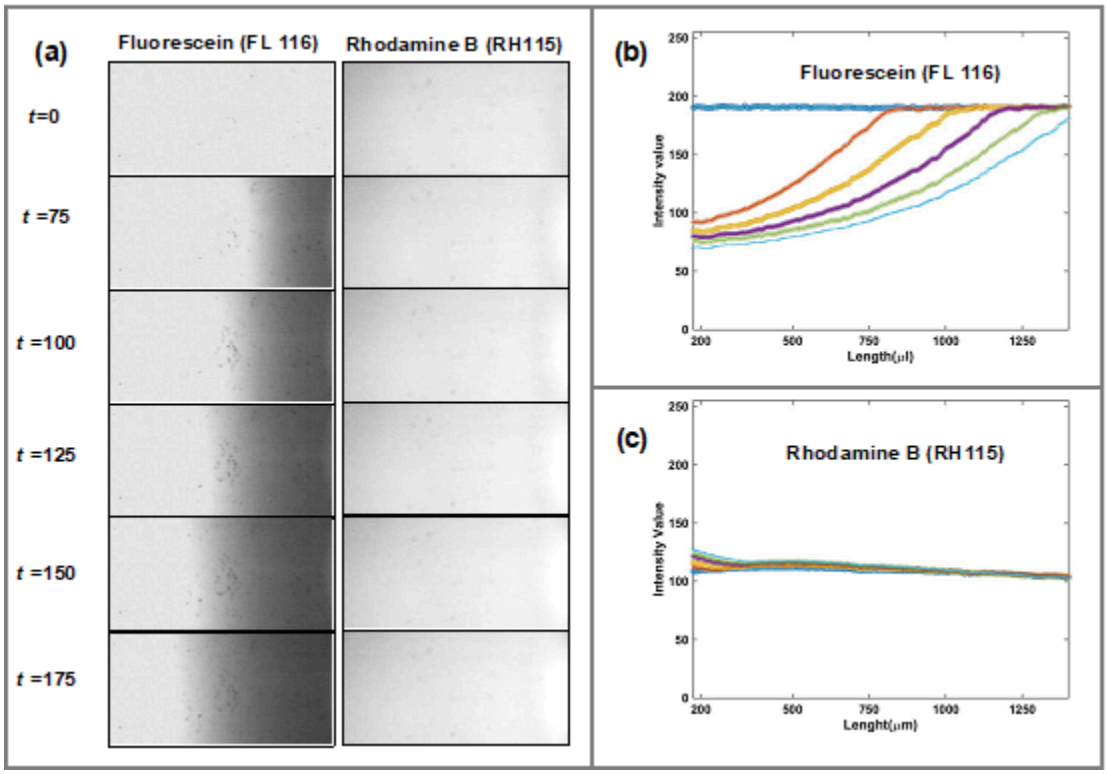


Figure 6

pH-sensitive emissions of rhodamine B and fluorescein dyes (a) Greyscale images at 6 different time steps during CO_2 dissolution in fluorescein and rhodamine B dyes in DI water in a PMMA microchannel (b, c) Time-dependent emission profiles along the channel for (b) fluorescein and (c) rhodamine B.

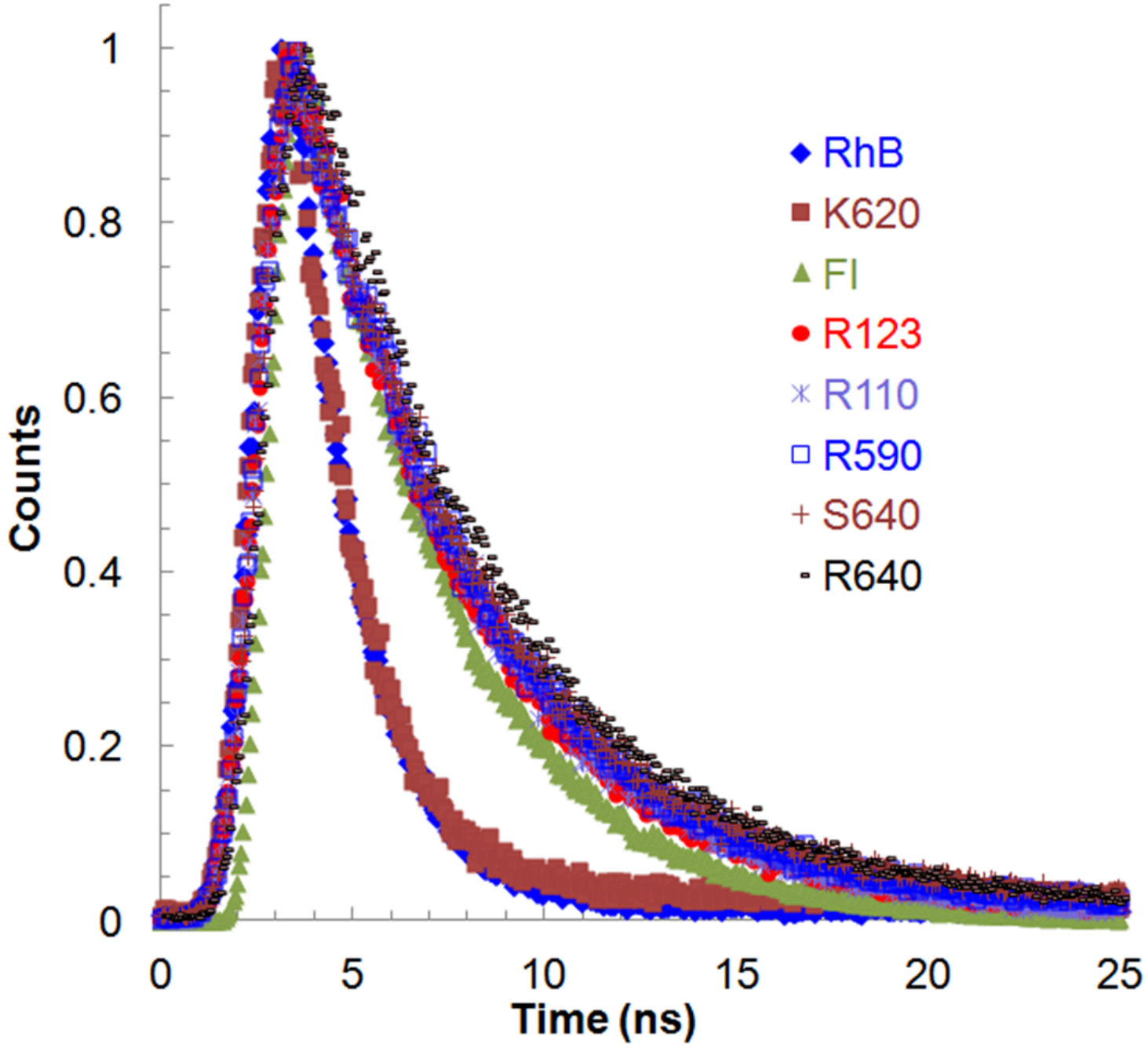


Figure 7

Fluorescence lifetime measurements for Fl and seven water-soluble rhodamine dyes.

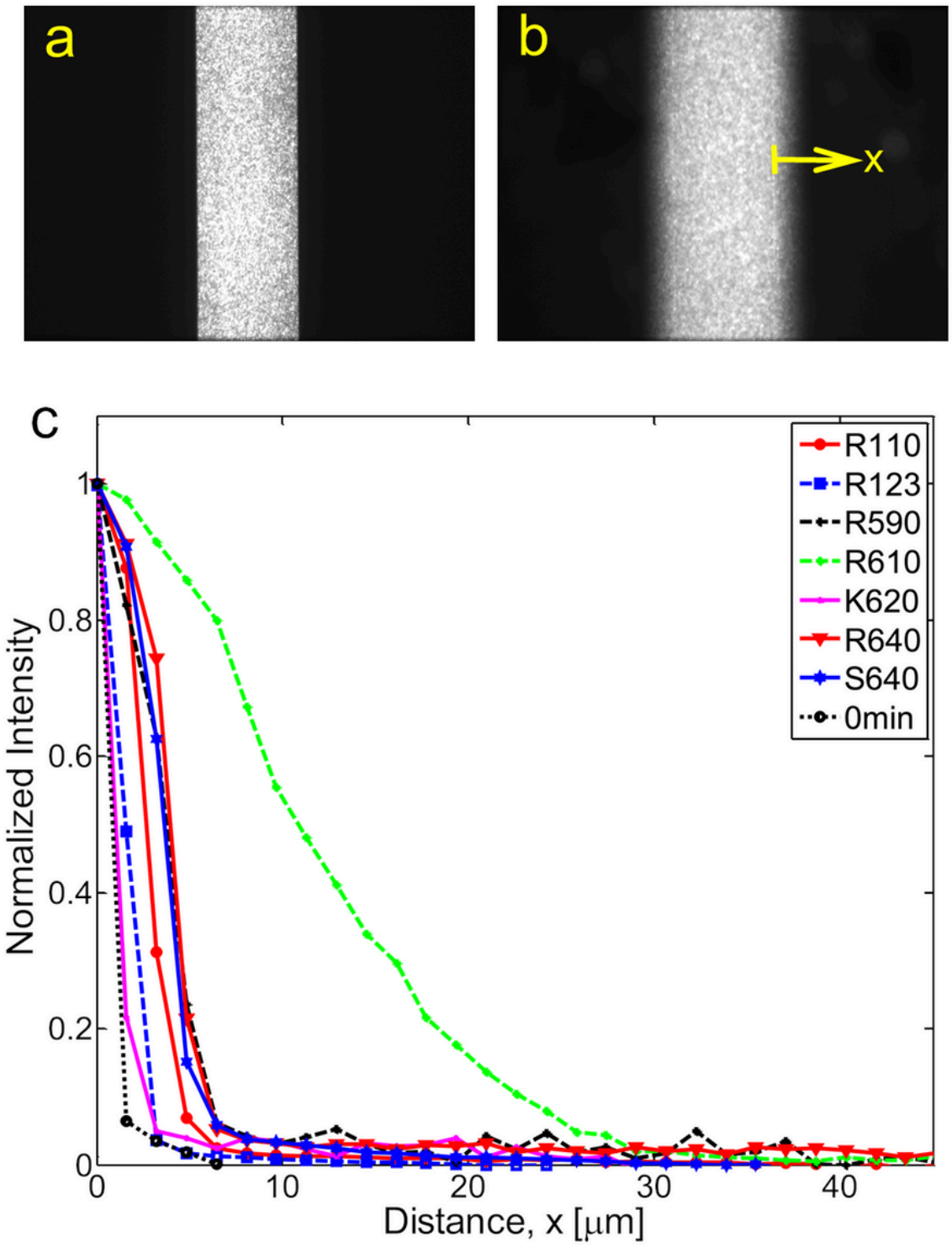


Figure 8

Greyscale channel images at 0 min (a) and 60 min (b) after RhB was injected. (c) Intensity profiles at 60 min of injection along the line in (b) from the PDMS-water interface.