



HAL
open science

Spectral characterization of fluorescein species in aqueous solution for laser-induced fluorescence thermometry

Lionel Perrin, Gilles Ledoux

► **To cite this version:**

Lionel Perrin, Gilles Ledoux. Spectral characterization of fluorescein species in aqueous solution for laser-induced fluorescence thermometry. *Experiments in Fluids*, 2023, 64 (6), pp.117. 10.1007/s00348-023-03632-9. hal-04145751

HAL Id: hal-04145751

<https://hal.science/hal-04145751>

Submitted on 30 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Spectral Characterization of Fluorescein Species in Aqueous Solution for Laser-Induced Fluorescence Thermometry

Lionel Perrin* and Gilles Ledoux

Institut Lumière Matière, Université Claude Bernard Lyon 1, 69622 Villeurbanne, France

E-mail*: l.perrin88@gmail.com

Keywords: Thermometry, Laser-induced Fluorescence, Liquid, Temperature sensitivity, Fluorescein

Abstract

Measurement of liquid temperature in single or multiphase systems is a topic of high interest for fluid mechanics in order to have a better understanding of heat exchange and phase changes in many physical problems as well as for improving industrial processes. Laser-induced Fluorescence Thermometry (LIF-T) is a powerful tool to investigate liquid temperature in permanent or transient conditions due to the fast fluorescence response of the technique. By diluting a fluorescent dye sensible to temperature in a liquid and after laser excitation of this solution at a specific and known wavelength, fluorescence is emitted by the dye almost instantaneously and could be isolated as a function of the temperature of the dye thanks to a ratiometric method. In this work, two fluoresceins commonly used as fluorescent dyes were spectrally studied in water at excitation wavelengths of 473 and 532 nm. Fluorescein Disodium and Fluorescein 27 were chosen as they present positive and high temperature-sensitivities as well as low toxicity. Also, systematic calibrations between -10 and 80°C under liquid state are reported for aqueous solutions. Results are compared to available data from the literature and temperature-sensitivity is discussed in relation with pH, excitation wavelength and temperature range in order to extend the knowledge on the topic and for clarification regarding some confusion found in the literature. Finally, we proposed an improvement of a method analyzing the sensitivity as a function of the wavelength for a better approach in the process of selecting spectral bands for LIF-T applications. Following this method, two examples of practical application are given for dye and spectral band selection depending on the constraints of experimental measurement.

1 Introduction

For many years, a certain amount of fluorescent organic molecules like Fluorescein species have been used for their interesting spectral properties related to temperature measurement as well as other parameters in liquid flow. Methods like Laser induced fluorescence thermometry (LIF-T) have been developed over decades thanks to these types of molecules to measure average or local temperature in monophasic and multiphasic systems (Ichiyanagi et al. 2007; Perrin et al. 2015). Nowadays, many non-invasive techniques exist to perform temperature measurement in liquid but LIF technique presents a lot of advantages. Thanks to its fast acquisition time, it eases the measurement especially for temperature mapping at fast flow rate at the difference of Raman Spectroscopy (Ewinger et al. 2013; Podbevsek 2019) or Fluorescence Lifetime Method (Okabe et al. 2012; Wang et al. 2022) which require longer time exposure and powerful lasers to get enough signal in order to maintain good temperature accuracy. LIF technique is also versatile in enabling the study of pure liquid as well as multiphasic flow including spray but also cavitation unlike Rainbow Refractometry (Wu et al. 2018) which is limited to the study of droplet.

The progress made in thermal characterization for macro and micro-flow challenges the application of these techniques. Indeed the spectral properties of these fluorescent dyes should be characterized in experimental conditions that are more and more difficult in practice. For example, improvement in increasing the temperature sensitivity to reach better measurement precision is always a topic of research and is affected by many parameters to control like the temperature range, pH, wavelength of excitation or the use of one or a mixture of two fluorescent dyes. In the next sections, the progress made over the past years on the two main fluorescein molecules used in water for LIF thermometry, i.e. Fluorescein Disodium (FI-Dis) and Fluorescein 27 (FI-27), will be summarized. Although Fluorescein species are also miscible in alcohol where they could be used for the same purpose, we will focus in our study on the case of aqueous solution only.

1.1 pH sensitivity of Fluoresceins

For organic molecules, pH is a critical parameter regarding its influence on absorption and emission spectra. A few works were published on pH-dependence of FI-Dis but not as much was accomplished in regards to FI-27. Leonhardt et al. (1971) studied the effect of pH on the fluorescence of FI-Dis and FI-27 with a UV excitation wavelength of 365 nm. The absorption spectra for FI-27 is strongly influenced by the pH with a significant change of spectral shape and an increase of the intensity of the peak value when increasing the pH. For both fluoresceins, the intensity of the emission spectrum is increasing with pH under this excitation wavelength over the whole range between 2.75 and 12 pH units. They also reported that FI-27 solubility fell below 10^{-5} M for strongly acidic pH (between 3 et 1). For FI-Dis, Coppeta and Rogers (1998) also observed an increase of the absorption coefficient over the whole spectrum when increasing pH from 6 to 9. Many authors (Ichiyanagi et al. 2007, 2009, 2012b, a; Hishida et al. 2014) used the good ability of FI-Dis to vary with pH between 6 and 7.7 units to evaluate the mixing efficiency of electro-osmotic flow for solutions with different ion concentrations and to measure the concentration of dissolved CO₂ gas that impacts as well the pH of the solution in a T-shaped microchannel. They used a continuous-wave Ar-ion laser as a source of excitation of 488 nm with an energy of 50 mW and the dye was dissolved at 10^{-4} M in water.

Walker (1987) compared the fluorescence intensity of FI-Dis to absorption measurements of Lindqvist (1960) as a function of pH. They observed almost superimposed normalized curves showing a strong impact of the pH on

fluorescence with a strong increase from 4 to 8 units where it reaches a plateau. Chaze (2017) obtained the same observations adding the effect of temperature on the fluorescence intensity over the whole spectrum at 30 and 60°C. They observed a positive temperature sensitivity for each pH with the highest sensitivity for pH = 5.6. Doughty (2010) studied at ambient temperature the influence of pH on the absorption and emission spectra for FI-Dis. Their range of pH varied from 4.0 to 10.0 and they observed the same evolution using an excitation wavelength of 490 nm. They also studied the impact of the concentration with values going from 5.10^{-5} M to $1.6.10^{-6}$ M, which include the concentration of this present work. Chaze et al. (2016) reported very valuable results showing the positive temperature-evolution and negative pH-evolution of the absorption coefficient for FI-Dis at 10^{-4} M between 25 and 80°C.

As a result, absorption properties of FI-Dis and FI-27 are relatively well known at ambient temperature through a wide range of pH. Regarding the fluorescence, as the excitation wavelength is another parameter to take into account, results are rather spread out in the literature with excitation going from 365 to 532 nm, pH values not always well referred to and focus on FI-Dis mainly.

1.2 Temperature-Sensitivity of fluoresceins

To determine the sensitivity, two different methods can be found in the literature. One can apply the ratio between the intensity of fluorescence at two different temperatures. In this case, the whole spectrum, or only a portion of it, can be used in the calculation (Lemoine et al. 1999). This is called the one color LIF (1c-LIF) method. But the concentration of the fluorescent dye, the intensity of the incident laser and the volume of detection of fluorescence must remain constant during the experiment or be quantified, which is very difficult if not impossible in a lot of cases as for multiphasic measurements. Lavieille et al. (2001) developed the two color LIF (2c-LIF) method which is based on the calculation of a ratio of fluorescence between two spectral bands of the same spectrum. This allows to suppress the effect of the previous factors on the fluorescence signal and to isolate directly the temperature as a function of the fluorescence thanks to a reference taken at a controlled temperature before the experiment.

A dye might present a positive or a negative temperature-sensitivity and Sutton et al. (2008) proposed a mixtures of these two types of dye to increase the global sensitivity in the case of 2c-LIF. Most commonly used dyes, as Rhodamine B or Kiton Red 620, present a negative temperature sensitivity under classically used wavelengths of excitation ($\lambda_{exc} = 473, 488, 514$ and 532 nm). So it is important to further study Fluorescein species where their negative or positive natures in terms of temperature-sensitivity are both present under classical excitation wavelengths. Indeed, regarding FI-Dis, Coppeta and Rogers (1998) observed a slightly negative dependence for $\lambda_{exc} = 488$ nm and positive dependence for $\lambda_{exc} = 514$ nm. Walker (1987) confirmed the previous temperature sensitivity for $\lambda_{exc} = 488$ nm. For FI-27, Sutton et al. (2008) showed a positive temperature sensitivity under an excitation of 532 nm. It has to be noted that this sensitivity is calculated over the whole fluorescence spectrum and should be regarded as a preliminary result to observe the potential of temperature-sensitivity. Further analysis should be done for direct application as only a part of the fluorescence spectrum might be used for temperature measurement considering that the sensitivity depends on the wavelength.

As seen before, little is known regarding the influence of pH on the fluorescence of FI-27. It is nonetheless possible to get some insights thanks to the work of Estrada-Pérez et al. (2011) who studied the temperature sensitivity of the mixture FI-27 and Rhodamine B under various excitation wavelengths (488, 510, 514, 526 and

532 nm). Even though they didn't study each dye separately, the contribution of each dye to the entire fluorescent spectrum could be analyzed quantitatively. As reported in Tab. 1 for the two fluoresceins, they also observe a negative temperature-sensitivity for FI-27 at $\lambda_{\text{exc}} = 488$ nm and a positive at $\lambda_{\text{exc}} = 526$ and 532 nm. $\lambda_{\text{exc}} = 510$ and 514 nm are considered as a transition regime. In other studies and ours, the sensitivity remains negative but very low for $\lambda_{\text{exc}} = 473$ and 488 nm for both fluoresceins. Also FI-Dis has clearly a positive temperature sensitivity for $\lambda_{\text{exc}} = 514$ nm which is not as evident for FI-27. Estrada-Pérez et al. (2011) observed a dependence of temperature sensitivity to pH for their mixture but only used pH = 5 or 11 and the authors attributed it to FI-27 only. These insights are valuable as it is very difficult to find information regarding the influence of pH on the fluorescence of FI-27. Indeed, the pH is almost never mentioned regarding studies on this last dye (Sutton et al. 2008; Dunand et al. 2012; Chaze et al. 2016).

Chaze et al. (2016) showed that FI-Dis has higher positive temperature sensitivity than FI-27 between 27 and 80°C when excited at $\lambda_{\text{exc}} = 532$ nm but at pH = 5.6 for FI-Dis and an unspecified value for FI-27 as reported in Tab. 1. It has to be noted that the equation used to obtain the temperature sensitivity is different between Sutton et al. (2008) and Chaze et al. (2016) so that direct comparison is not possible regarding the temperature sensitivity of FI-27. Clarification of the sensitivity calculation and comparison will be brought in the Experimental Set-up Section.

In summary, FI-27 and FI-Dis present a negative and very low temperature-sensitivity when excited at 473 or 488 nm and a positive temperature-sensitivity when excited at and above 514 nm (Sutton et al. 2008; Estrada-Pérez et al. 2011; Chaze et al. 2016) (see Tab. 1). Many authors agreed on the impact of the absorption coefficient to describe the positive-temperature sensitivities. For example, Sutton et al. (2008) showed that, for FI-27 with $\lambda_{\text{exc}} = 532$ nm, the positive sensitivity to temperature is explained by the change of absorption coefficient with temperature and is independent of the fluorescence quantum yield. Many authors (Sjöback et al. 1995; M. Baruah et al. 2006; D.L. Silva et al. 2010; Chen et al. 2012; Panchompoo et al. 2012; Chaze et al. 2016) showed the impact of the temperature in changing the absorption coefficient and that this evolution is also a function of the pH. They also explained that different protolytic forms of fluorescein might appear at different pH and modify the emission spectrum as well. Indeed, Sjöback et al. (1995) observed four ionic forms of FI (FH_3^+ , FH_2 , FH^- and F^{2-}) in aqueous solution and each are more or less present as a function of the pH. The cation and neutral form show a quantum yield close to 0 and the anion and dianion have a quantum yield of 0.37 and 0.93 respectively.

1.3 Temperature calibration for water in the case of undercooling

From our laboratory, non-published results in the case of water for cavitating flows in microsystem seem to indicate that undercooling can appear in certain conditions with high flow rate. As a consequence, to study precisely this type of physical phenomenon constitutes a strong motivation in obtaining calibrations which go as low as possible in terms of temperature. Up to now, only a few works have been published on temperature measurement close to or under 0°C for aqueous solution in liquid state using LIF. Water would normally ice at 0°C at ambient pressure but metastable state might be observed and complex aqueous solutions might induce a shift of icing to lower temperature. Shafii et al. (2010) used a mixture of FI-Dis and Kiton Red 620 (KR620) excited at 514 nm in water to study the solidification front at the macroscale. They used NaOH to get pH = 7.8 in order to have pH-independence of their solution. They also used ammonium chloride (NH_4Cl) at the

concentration of 19 %wt to avoid icing above -15°C in water but their calibration range of temperature was between 4 and 20°C only. To the best of our knowledge, only Stiti et al. (2019) reported a calibration as well as temperature measurement in a droplet with temperature below 0°C for liquid state. They used Rhodamine 560 (Rh560) having positive temperature sensitivity and KR620 having negative sensitivity with $\lambda_{\text{exc}} = 532$ nm and a concentration of 5.10^{-5} M and 5.10^{-6} M respectively. The small concentrations allowed them to neglect the reabsorption effect for a typical optical path of 400 μm of their drops. They did a calibration on a suspended drop reaching down to -17°C before observing ice formation. They also observed that the fluorescence intensity dropped for the part of the spectrum corresponding to Rh560 meanwhile it remains very similar for the KR620 part, thus allowing for clear identification when solidification occurred. To confirm the undercooling of the droplet, they reproduced the calibration in a cuvette and observed similar results down to -5°C where this last experiment stopped.

In the present work, attempts for reaching temperature below 0°C with aqueous liquid phase were done successfully. The experimental procedure and its validation for obtaining these temperature calibrations will be described and discussed in the next sections. The aim of our study is to produce a complete report on Fluorescein species for LIF-T as they present very interesting properties in terms of temperature-sensitivity and spectral emission range. Regarding spectral characterization, the goal of this work is double. First, there is a need for temperature calibrations going to temperatures below 0°C for liquid state to be able to observe undercooling in aqueous liquid flow. It seems also necessary to precise some important experimental features in order to avoid some confusion found in the literature regarding pH and sensitivity calculation for example. In this work, we studied two dyes potentially having a positive sensitivity to temperature (Fluorescein Disodium and Fluorescein 27). Thanks to an experimental set-up allowing us to obtain liquid water under 0°C , each dyes were systematically studied between -10 and 80°C under excitation wavelengths of 473 and 532 nm. Temperature sensitivity will be evaluated and compared with available data from the literature. The choice of pH being crucial, it will be discussed all along this paper among other experimental conditions. Finally, a discussion is advanced regarding the methods used for spectral band selection and an improvement of the method of Chaze et al. (2016) is proposed regarding the characterization of temperature-sensitivity as a function of the wavelengths to facilitate this process that could be also adapted for pH or concentration-sensitivity characterization.

2 Experimental set-up and methodology

2.1 Experimental set-up

In our experiments, spectral study was done in a cell with a depth of 2 mm and a diameter of 1.4 cm containing around 0.3 ml of solution. Demineralized water with a resistivity of 10 $\text{M}\Omega\cdot\text{cm}$ from an Elga micromeg deionizer was used as solvent. Regarding the fluorescence study, Fig. 1 shows the different components of the experimental set-up. The cell containing the solution is placed inside a Linkam HFS600 set-up which allows us to control its temperature and rate of heating or cooling. The cell is filled entirely with the solution and covered by a thin glass plate to prevent evaporation. It is then placed on a thermostat and enclosed in a small chamber to prevent heat exchange and convection with ambient air. When the thermostat is set at a specific temperature, a delay of two minutes is necessary to reach temperature equilibrium in the cell. Spectra were taken two and five minutes after the thermostat reached equilibrium at each temperature to validate the measurement. With this

specific set-up, spectra were taken between 80°C and -10°C under liquid state with a step of 10°C and are seen to be reproducible.

Here, we assume that the temperature of the liquid is directly connected to the temperature of the thermostat as we cannot measure it with a thermocouple. It has to be noted that differences can automatically occur between the temperature of the thermostat and the one of the cell which could increase when reaching very low temperature, especially below 0°C. In order to reduce this difference as much as possible, an efficient thermal transfer between the thermostat and the cell containing the liquid had to be put into place. The cell, in sapphire, presents a good thermal conductivity and the plate supporting the cell is made of silver which has a very high thermal conductivity. Also, the main part of the thermostat is made of Aluminum oxide which has a rather high conductivity for a ceramic material, similar to stainless steel. Finally, the experiments described in Section 2.2 and Section 2.3 seem to confirm that temperature differences between the thermostat and the liquid are negligible.

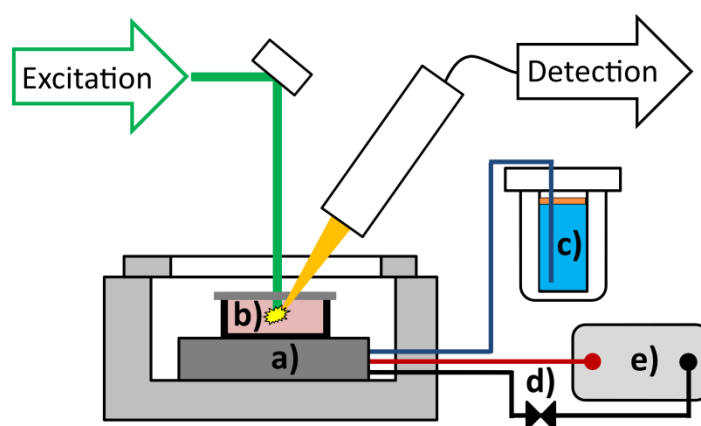


Fig. 1 : Scheme of the experimental set-up for temperature calibration with a) Linkam HFS600 system with liquid nitrogen supply and electrical resistive heater, b) solution in sapphire cell covered by glass, c) liquid nitrogen, d) liquid nitrogen pump, e) temperature control system.

The excitation sources are composed of a Xenon lamp with a power of 25 μ W at 473 nm and a Nd:Yag laser in continuous mode at 532 nm with a power of 50 mW where we applied a filter with an OD of 3 for this last one. The luminescence signal is collected by a bunch of optical fibers (ANDOR SR-OP-8002) and fed through a f-matcher to an Andor Kymera 328i spectrograph equipped with an internal shutter for automatic background subtraction and coupled to an ANDOR Newton DU970P EMCCD. Regarding the measurements, the grating used was 300 grooves/mm blazed at 500 nm and the measured resolution was 1.6 nm.

Parameters of the experiments like excitation intensity, dye concentration and optical path are to be controlled in order to allow a good signal to noise ratio (SNR) regarding the fluorescence without having too much re-absorption of fluorescence, saturation of fluorescence or photobleaching. In our case, along the optical path of fluorescence with the concentration used, the optical density was calculated to be much lower than 0.1 over the spectra of emission (~ 0.01 at the maximum of absorption). Regarding the saturation of fluorescence, this regime occurs when the intensity of fluorescence is no more correlated linearly to the excitation intensity which would lead to a dependence of temperature sensitivity to laser irradiance. Chaze et al. (2016) have evaluated the upper limit of linear regime at a few 10^5 $W.m^{-2}$ for FI-Dis and RhB. In our case, saturation of fluorescence is easily

prevented as laser irradiance is estimated to be lower by several order of magnitude ($<10^1 \text{ W.m}^{-2}$). Finally, discussions about photobleaching are provided with preliminary experiments in Section 2.3.

2.2 Metastability

When we decrease the temperature of the solution, the systematic undercooling we observed might be explained by freezing-point depression where the addition of a non-volatile solute in water like our dyes could lead to a much lower temperature for icing to occur (Ramzay 1949). But measurements with deionized and filtered water showed the exact same behavior with ice forming below -10°C and melting occurring only when warming back to 0°C so that the impact of our solute seems negligible on water phase change in our experiments. The conditions of experiments themselves might be sufficient to explain this behavior with the use of deionized and filtered water so that the amount of nucleus, facilitating the icing, is limited to a minimum. In this case homogeneous nucleation, that would happened in the liquid phase far from the surfaces, is prevented. Also, the nature of the surfaces of the container being sapphire and glass are relatively smooth and were cleaned with ethanol and dried before use, thus limiting the other type of nucleation, i.e. heterogeneous nucleation, that could appear at the interface between our liquid and the two different surfaces (sapphire and glass). Furthermore, these surfaces are known to be hydrophilic which help to stabilize the liquid phase. Heterogeneous nucleation is usually easier to obtain as nuclei present at an interface have lower energy barrier than nuclei inside the liquid. Finally, it is known that small volumes make it easier to observe undercooling but that melting almost always occurs at 0°C which is the case in our experiments. This last observation is reassuring regarding concerns about temperature differences between the thermostat and our solution as expressed in the previous section.

All these reasons might explain the metastable state below 0°C that we observed in a reproducible way for all the different solutions we used in this work.

2.3 Stability of the chemical and photo-physical properties of the dyes in our experimental set-up

Preliminary experiments on Rhodamine B (RhB), at 10^{-5} M and under an excitation wavelength of 532 nm, were done to assess the stability of the solution in terms of chemical and photo-physical properties as many physical phenomena are into play (Demchenko 2020). Indeed, photobleaching of the dye might happen due to laser excitation and degradation of the dye can occur due to temperature settings of the experiments as well as temperature conditioning. Simultaneously, these preliminary results allow us to test the capacity of our experimental set-up in producing reliable temperature calibrations and to assess the different settings that we will use in order to obtain thermal equilibrium and proper fluorescence measurements at different temperatures. Emphasis has been put on undercooling situations as we used only one experimental set-up at the difference of (Stiti et al. 2019) who confirmed their calibrations below 0°C thanks to two experimental set-up.

In Fig. 2, a ratio of fluorescence for RhB was taken at different temperatures through a single experiment during more than 5h. The excitation was discontinuous and only set for measurement with a duration of the order of 5 s. The ratio of fluorescence corresponds to the sum of intensity signal between 580-600 nm divided by the sum between 600-800 nm. For comparison purposes, ratios of fluorescence are normalized by the mean value for 10°C in a) and by the first value of each temperature in b). The solution was set at 10°C for 20 minutes before measurement, then at 0°C , -5°C and -10°C for 10 minutes before measurement to allow thermal equilibrium. The rate of cooling was relatively slow with $1^\circ\text{C}/\text{min}$ in order to favor undercooling. In Fig. 2 a), the variation of the

ratio for each temperature was less than 0.3% over at least 100 min, showing a strong stability of our measurements as it presents no photo-bleaching over the time of excitation and simultaneously no temperature degradation of the dye due to temperature change in our experimental conditions.

As a regular calibration takes around 1h30, it is important to ensure that the solution remain photo-stable for a much longer period of time. Also, we observe that thermal equilibrium is reached in two minutes only, showing that the parts of the set-up that might still change its temperature after two minutes, especially the air surrounding the cell, don't impact much the temperature of our solution.

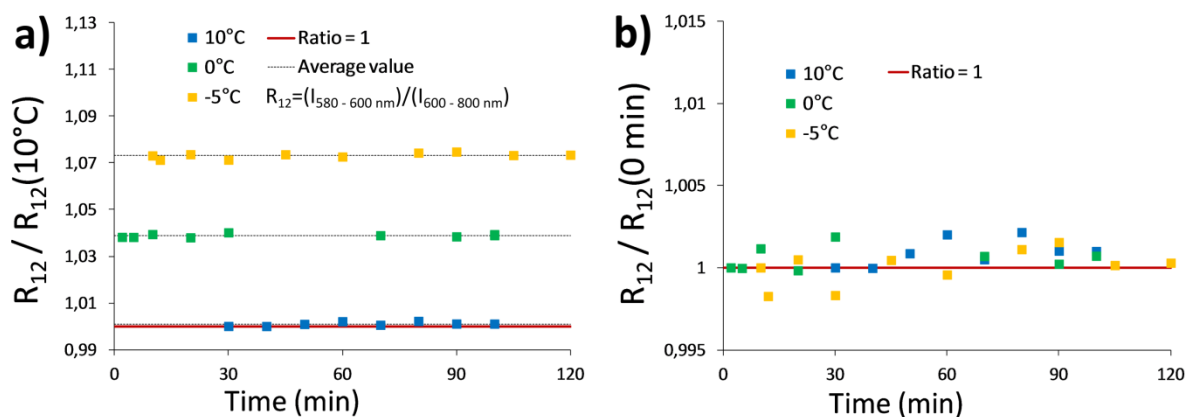


Fig. 2 : Measurement over time of a ratio of fluorescence at different temperatures with discontinuous excitation for RhB normalized by the value at 10°C in a) and normalized by the initial value at each temperature in b).

A second experiment was conducted to assess the potential of supercooling of the solutions in our experimental set-up. The solution was set at the temperature of 20°C and cooled down to -15°C where icing occurred rapidly. In between, the thermostat was set at 10°C, 0°C, -5°C and -10°C for at least 50 min each in a single experiment. Fluorescence spectra were taken regularly every 15 to 20 min. As they remain very similar at a defined temperature, only the first spectrum taken after 5 min and the last were shown on Fig. 3 a) for clear comparison. A stable fluorescence spectrum is observed after 2 min only and remains the same after 50 min showing that the evaporation is negligible when going below 20°C even after a long time.

For RhB, the intensity of the signal increases when decreasing the temperature. Then, reaching 0°C, the spectrum is the same even after 100 min showing no icing of the solution. When going down to -5 and -10°C for close to an hour each, no icing is observed either. Only when going below -10°C, the liquid turned to ice but only after 60 min which was clearly visible by eye and by the dramatic decrease of fluorescence intensity close to the noise level. For each temperature in Fig. 3 b), the spectra taken over time were normalized following the same method as in Fig. 2 b). The variation of the ratio was less than 0.3% in every situation. These results showed that, with this experimental set-up, temperature calibration can be easily produced down to -10°C thanks to the surprising stability of the liquid state under 0°C and fast reaching of a stationary signal measurement (~2 min for each point). Finally, it has to be noted that the change of peak intensity seems linear with the temperature evolution which comforts us regarding the precision of the temperature measurement.

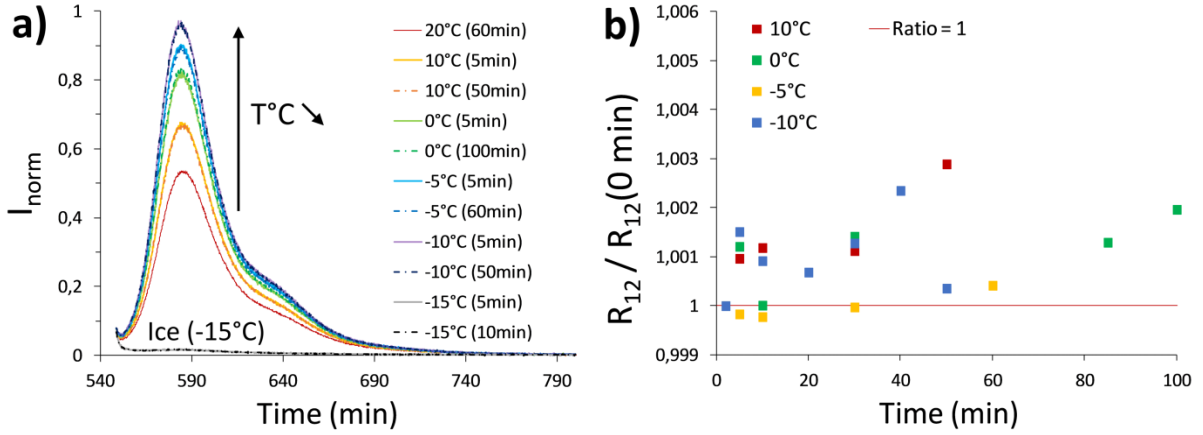


Fig. 3 : Normalized fluorescence spectra at different $T^{\circ}C$ over time for RhB under in a) and normalized ratio of fluorescence in b).

These experiments confirmed that we were able to prevent as much as possible every aspects that could be detrimental to fluorescence measurements : the thermal stability of our set-up and of the dye, the photo-stability under discontinuous but numerous laser excitations for a duration far above the one needed for our typical calibration measurement.

2.4 Evaluation of Temperature Sensitivity

In this section, it is important to precise the calculation method to determine the temperature sensitivity S in $\% \cdot C^{-1}$. In the literature, the function below is commonly used (Walker 1987; Coppeta and Rogers 1998; Sutton et al. 2008):

$$S = \frac{I(T_2) - I(T_1)}{T_2 - T_1} * 100 \quad (1)$$

$I(T)$ corresponds to the sum of intensity of fluorescence over the whole spectrum at a specific temperature T . The main drawback of this function is that the given value of sensitivity depends on the temperature reference $R(T_1)$ which sometimes makes the comparison not adequate between studies. Chaze et al. (2016) proposed another function that is independent from this temperature and described as below:

$$S = \frac{100 * \ln(I(T_2)/I(T_1))}{T_2 - T_1} \quad (2)$$

These two functions give different values and shall not be mixed together. For comparison purposes, all values taken from the literature using one of these functions were systematically recalculated with the other and both results are reported in Tab. 1. It also has to be clarified that these two functions are calculated over the whole spectrum which presents certain limitations. Indeed, to be a correct form of comparison, it assumes that the change of intensity of the emission spectrum is homogenous at all wavelengths. But if only a part of the spectrum has a temperature sensitivity and another part has no or an opposite sensitivity, then the calculation will result in lower and biased global sensitivity making it unfit for precise comparison. As a consequence, the S value allows us only to consider the potential of a dye but deeper analysis shall be made to get the most of it in practical situations. For this reason, the commonly used function Beta $\beta(\lambda)$, giving the relative sensitivity as a

function of the emission wavelength, will be systematically added for better understanding of the temperature sensitivity of each dye. From the spectra of fluorescence at two different temperatures T_1 and T_2 , it is possible to evaluate this function $\beta(\lambda)$ described as follow:

$$\beta(\lambda) = \ln \frac{I_1(\lambda, T_1)}{I_2(\lambda, T_2)} / \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \quad (3)$$

Each calculated value of the temperature sensitivity is subtracted by the maximum and is represented in Tab. 2 for direct comparison between cases:

$$\Delta\beta(\lambda) = \beta(\lambda) - \beta(\lambda_{\max}) \quad (4)$$

In the next sections, the use of the Beta function will always refer to this last equation Delta Beta.

3 Experimental results and discussion

3.1 Absorption spectra

Absorption spectra were measured thanks to a Perkin Elmer lambda900 UV/Vis/NIR spectrometer. On Fig. 4, the normalized coefficients of molar extinction ϵ measured as a function of the wavelength λ are represented for FI-27 and FI-Dis at 10^{-5} M with pH = 5.7, 7.0 and 9.0. Wavelengths were studied between 250 and 800 nm and data are represented between 350 and 600 nm for convenience. The wavelengths of excitation that will be used later in this study, i.e. 473 and 532 nm, are also superimposed.

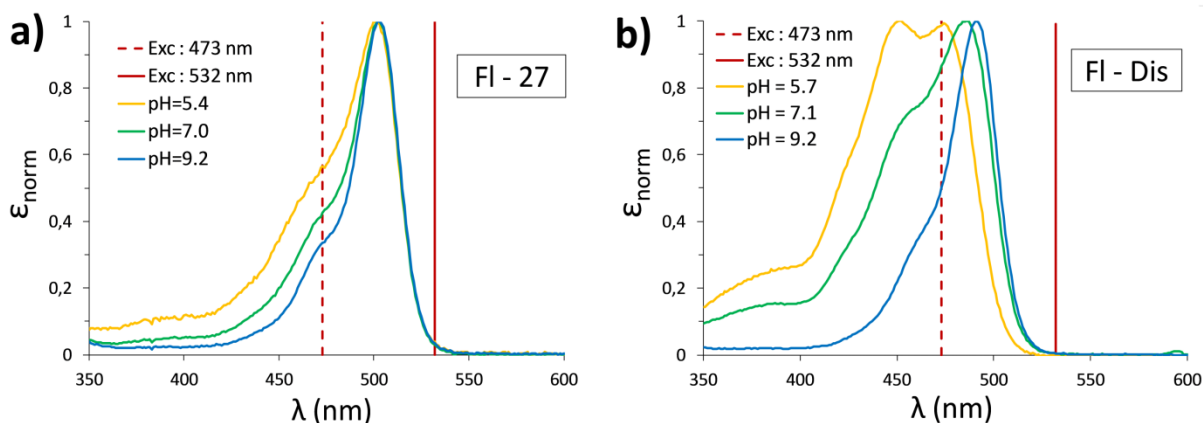


Fig. 4 : Normalized coefficient of molar extinction ϵ as a function of the wavelength λ in nm for FI-27 in a) and FI-Dis in b) at three different pH. In dashed and full lines are represented the wavelengths of excitation of 473 nm and 532 nm respectively.

We can see that the absorption coefficient is strongly influenced by the pH of the solution. For FI-Dis, the spectrum at pH = 5.7 shows two peaks. For higher pH, a single peak is observed and is translated to higher wavelengths with a narrow spectrum. For FI-27, a peak is observed between 500 and 505 nm and does not change between a pH of 5.4 and 9.2 units but the whole shape of the spectrum is proportionally flattened when decreasing the pH. Regarding the excitation wavelength, $\lambda_{\text{exc}} = 473$ nm is more in agreement with the absorption spectra of the two dyes and especially with FI-Dis at pH = 5.7. At $\lambda_{\text{exc}} = 532$ nm, the superimposition with the spectra is very low for FI-27 and even worse for FI-Dis. Nonetheless, previous publications (Sutton et al. 2008; Chaze et al. 2016) showed that it is possible to use this excitation wavelength for these two dyes under the

condition of using higher concentration and/or higher excitation intensity which may have some detrimental effects regarding the saturation of the signal and spectral conflicts between the absorption and excitation spectra. The next section on emission spectra will confirm the possibility and the interest of studying the two fluoresceins under 532 nm as an excitation wavelength also.

3.2 Emission spectra

Previous studies showed that Fluorescein 27 (FI-27) and Fluorescein Disodium (FI-Dis) are good candidates for LIF-T in aqueous solutions even if FI-27 has a lower water solubility. In practice, this last characteristic makes it harder to dissolve the dye completely especially if the final concentration used for experiments is near the maximum of solubility. Sonication is often required for complete dissolution and regular checking of the stored solution has to be done in order to ensure that there is no precipitation of the dye over time. FI-27 and FI-Dis have a solubility in water of $5 \cdot 10^{-2} \text{ g.L}^{-1}$ and 40 g.L^{-1} leading to a maximum concentration of $1.5 \cdot 10^{-4} \text{ mol.L}^{-1}$ and $10^{-1} \text{ mol.L}^{-1}$ respectively. No problem of precipitation has been noticed in our case as we used a concentration of 10^{-5} M for each dye but care should be taken if one has to use a higher concentration, especially for FI-27.

The positive or negative nature of the temperature-sensitivity is controlled by the excitation wavelength as described in the introduction. In Fig. 5, we give an example of the spectral results with the superimposition of the normalized absorption and emission spectra at $\lambda_{\text{exc}} = 532 \text{ nm}$ for FI-Dis and FI-27 at ambient temperature and $\text{pH} = 9.2$. Here, we managed to obtain sufficient SNR with a concentration of 10^{-5} M for both dyes. Both spectra are slightly translated to the left for FI-Dis in comparison to FL-27. Unfortunately, with this excitation wavelength, an important part of the spectrum is cut by a Notch filter NF01-532U-25 from Semrock that we applied.

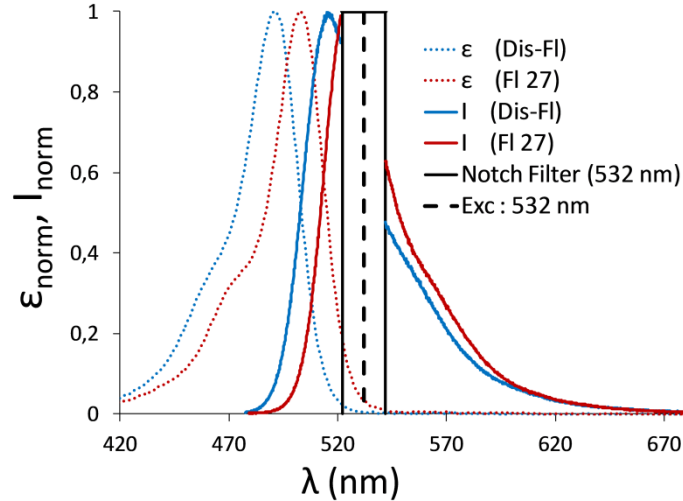


Fig. 5 : Normalized absorption coefficient ϵ_N and emission spectra I_N at $\lambda_{\text{exc}} = 532 \text{ nm}$ and $\text{pH} = 9.2$ for FI-Dis and FI-27 at 25°C and 20°C respectively.

3.2.1 Temperature sensitivity over the whole fluorescence spectrum

From the literature (Walker 1987; Chaze 2017), we know that the intensity of the absorption and emission spectra at ambient temperature is very low at acidic pH, then increases significantly with pH until reaching a plateau around $\text{pH} = 7.5-8$. We might wonder how the temperature sensitivity evolves at all pH and if it remains the same at pH higher than 8 when the fluorescence spectrum is not affected anymore by pH. To answer this question, we measured experimentally the temperature sensitivity between -10 and 80°C for FI-Dis at $\text{pH} = 5.6$,

6.8, 8, 9 and 10 and for Fl-27 at pH = 5.6, 7.0 and 9.0. At pH = 4, the signal was too low to get any measurement in both cases.

In Tab. 1, our results on Fl-Dis and Fl-27 are summarized. Comparable data from the literature are also reported for discussion. Information about concentration, pH, wavelength of excitation λ_{exc} and temperature range are reported when available. Despite having data going from -10 to 80°C for each of our experiments, we used temperature ranges in Tab. 1 that are comparable to the literature. The sensitivity was calculated following both Eq. (1) and (2) over the whole emission spectrum. This was made in order to clarify and compare directly the results on temperature-sensitivity from the literature to our values.

In all cases, the temperature sensitivity is slightly negative and very low when using $\lambda_{exc} = 473$ or 488 nm. Both Fluoresceins present an important positive temperature dependence when excited at $\lambda_{exc} = 532$ nm. At $\lambda_{exc} = 514$ nm, an interesting positive temperature sensitivity is also observed for Fl-Dis but no measurement on Fl-27 has been reported to our knowledge at this wavelength. Experimental data of the sensitivity as a function of the excitation wavelength are represented in Fig. 6.

Dye	Concentration	pH	Excitation (nm)	Temperature range (°C)	Sensitivity (%.°C ⁻¹) Eq. (1) / (2)	Reference
Fl 27	10 ⁻⁵ M	5.9 9	473	20 – 80	-0.17 / -0.18 -0.13 / -0.14	Present study
	5.92.10 ⁻⁷ M	7 ?	532	24 – 84	+3.50 / +1.89	Sutton et al. (2008)
	10 ⁻⁴ M	7 ?	532	27 - 84	+3.37 / +1.88	Chaze et al. (2016)
	10 ⁻⁵ M	5.6 7.0 9.0	532	20 – 80	+3.66 / +1.94 +3.48 / +1.88 +3.14 / +1.76	Present study
Fl-Dis	10 ⁻⁵ M	9	473	20 - 60 20 - 80	-0.19 / -0.20 -0.32 / -0.36	Present study
	?	10	488	20 – 61	-0.16 / -0.17	Coppeta and Rogers (1998)
	10 ⁻⁷ M	>9	488	24 - 46	-0.30 / -0.31	Walker (1987)
	?	10	514	20 – 61	+2.44 / +1.69	Coppeta and Rogers (1998)
	5.10 ⁻⁷ M	7.8	514	5 – 20	+1.93/2.28 (/5) +2.73/2.29 (/20)	Shafii et al. (2010)
	5.10 ⁻⁷ M	9.2	514	20 – 60	+2.45 / +1.71	Kim and Yoda (2010)
	10 ⁻⁴ M	5.6 10	532	27 – 84	+9.24 / +3.22 +5.29 / +2.44	Chaze et al. (2016)
	10 ⁻⁵ M	5.6 7.0 8 9 10	532	20 – 80 20 – 80 0 – 20 20 – 60 20 – 60	+6.31 / +2.61 +5.99 / +2.54 +3.63 / +2.73 +4.38 / +2.53 +4.43 / +2.55	Present study

Tab. 1 : Sensitivity over the whole spectrum of fluorescein dyes in the literature and the present study. Experimental details are added when available. Data in red were recalculated from original articles.

It is very interesting to compare our results on Fl-27 to those of Sutton et al. (2008) and Chaze et al. (2016) because the pH is unspecified in their publications. Using a similar temperature range (~20 to 80°C), we obtain the exact same sensitivity of +1.88 %.°C⁻¹ for pH = 7 which we assume is the pH they used then. It has to be

noted that the concentration is very different for each study and goes from $5 \cdot 10^{-7}$ M to 10^{-4} M but doesn't seem to impact the result which shows that there is a negligible impact of re-absorption in all these different studies.

We would also like to report that Chaze et al. (2016) and Chaze et al. (2017) had higher sensitivities than Sutton et al. (2008) although they claim the opposite. This is because they compare their values using Eq. (2) to the value of Sutton et al. (2008) who used Eq. (1). As a consequence, the study of Chaze et al. (2016) gave in fact the best average positive temperature-sensitivity in the literature to our knowledge. Similar to our remark on pH indication, it is very important to pay attention to the sensitivity calculation formulae to avoid any confusion.

We also brought new insights on FI-27 by studying the impact of pH on the temperature-sensitivity which was only roughly studied by Estrada-Pérez et al. (2011) where a higher pH leads to lower sensitivity in a two-dyes mixture with RhB. From our experimental results, we observed that the temperature-sensitivity is slightly decreasing with values going from +1.94, +1.88 and 1.76 $\% \cdot ^\circ\text{C}^{-1}$ for increasing pH of respectively 5.6, 7.0 and 9.0 with a temperature range of 20-80 $^\circ\text{C}$. For temperature range of -10 to 20 $^\circ\text{C}$, we obtained higher sensitivities and a slightly higher decrease of it with $S = 2.86, 2.55$ and $2.24 \% \cdot ^\circ\text{C}^{-1}$ for the same pH evolution.

Regarding FI-Dis, we found the same low negative temperature-sensitivity using 473 nm than Coppeta and Rogers (1998) and Walker (1987) at $\lambda_{\text{exc}} = 488$ nm for identical temperature ranges. This behavior is really similar between FI-Dis and FI-27. At higher excitation wavelengths, the temperature-dependence is always positive. At 514 nm, Kim and Yoda (2010) confirmed the good temperature-sensitivity of Coppeta and Rogers (1998) using a pH of 9.2 units instead of 10. Finally, using a similar pH (9-10), it is also important to point out the evolution of the sensitivity when using different λ_{exc} . Going from 473 nm to 532 nm and passing by 488 and 514 nm, lead to higher positive temperature-sensitivity when using pH-independent range. This behavior seems similar at lower pH corresponding to pH-dependent range for both fluoresceins, but can only be ascertained by our experimental results with $\lambda_{\text{exc}} = 473$ and 532 nm. At pH = 5.6, we found a lower value with $S = +2.61\% \cdot ^\circ\text{C}^{-1}$ than Chaze et al. (2016) who obtained $+3.22\% \cdot ^\circ\text{C}^{-1}$. We used a concentration that is ten times smaller but the other parameters are alike. It is difficult to explain this difference as, in the case of FI-27, we found the same results as Chaze et al. (2016), although the concentration we used is also 10 times smaller so that differences in concentration, experimental set-up or even protocol seem unlikely to be an explanation. Furthermore, as a higher concentration should lead to more significant reabsorption, and as the fluorescence signal increases with temperature for Fluorescein species with $\lambda_{\text{exc}} = 532$, then it should resolve in lower apparent sensitivity with the higher concentration of Chaze et al. (2016) which is the opposite of what is observed. Finally, the spectral conflicts are rather similar for the two Fluoresceins as shown in Fig. 5 so that if we find the same results for FI-27, differences between absorption and emission spectrum would hardly be an explanation either in the case of FI-Dis.

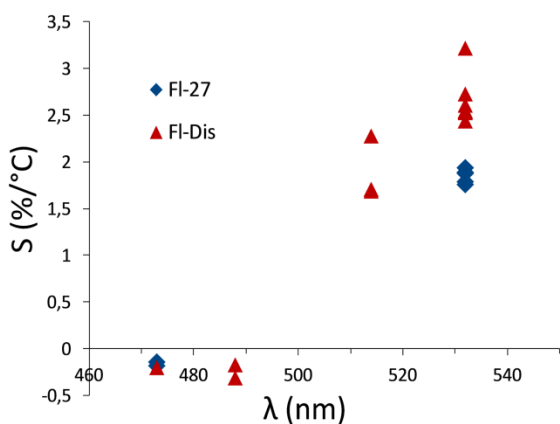


Fig. 6 : Temperature-sensitivity of FI-27 and FI-Dis for excitation wavelengths of 473, 488, 514 and 532 nm. Results come from the literature and our own experimental data.

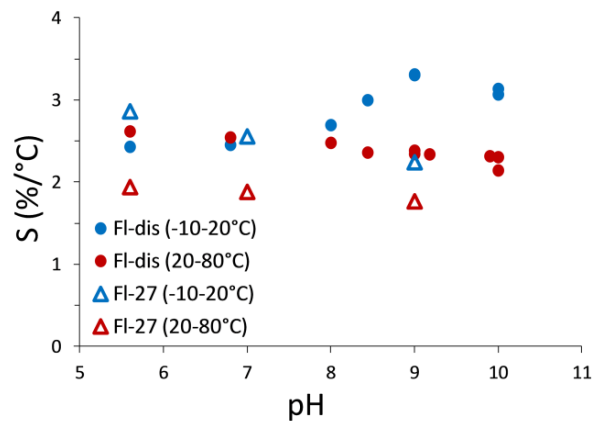


Fig. 7 : PH-dependence sensitivities of FI-Dis and FI-27 in at 10^{-5} M and excitation at 532 nm for two different temperature ranges.

In order to clearly see the impact of the pH on the temperature-sensitivity, our experimental results for both fluoresceins at $C = 10^{-5}$ M and at $\lambda_{exc} = 532$ nm are reported in Fig. 7 separated in two temperature ranges. Our experimental results show surprisingly that the sensitivity of FI-Dis over the whole spectrum between 20°C and 80°C is rather similar and decreases only slightly between pH = 5.6 and 10. Regarding the temperature range -10 to 20°C, the sensitivity is almost exactly the same as for the other temperature range with pH included between 5.6 and 8. At pH = 8, the sensitivity is increasing significantly until reaching a maximum at pH = 9, and finally a slightly lower value is found at pH = 10.

For FI-27, the sensitivity is always slightly decreasing when increasing the pH and the variation seems almost linear for both temperature ranges. $S(\lambda)$ is systematically higher for the lower temperature range. Finally, FI-27 has lower sensitivity than FI-Dis except at pH = 5,6 for the temperature range of -10 to 20°C.

From our experimental results and literature comparison, the most interesting dye is FI-Dis that has a positive temperature-sensitivity under an excitation wavelength of 532 nm as seen in Tab. 1. FI-Dis presents a higher sensitivity S averaged on the whole spectrum against FI-27 at any given pH except for pH = 5,6 over the temperature range of -10 to 20°C. Furthermore, the fluorescence spectrum of FI-Dis is more shifted to lower wavelengths (Fig. 5) which makes it easier to separate spectrally from another dye having a negative temperature-sensitivity that will be red-shifted regarding the fluorescence spectrum in the case of applying the Two dyes-Two colors/LIF technique (Chaze et al. 2017). From our study and to the best of our knowledge, it seems not possible to obtain a better positive or even absolute sensitivity than the one of Chaze et al. (2016) for the case of one dye among all the fluorescent dyes studied in the literature. Indeed, even Rhodamine B and Kiton Red 620, which show high negative sensitivities and are widely used, present lower values (around $-2,4 \text{ \%}\cdot\text{C}^{-1}$ for both (Chaze et al. 2016)).

We shall explain some drawbacks of using averaged values over the whole spectrum for comparison and estimation of temperature sensitivity. First of all, as pointed out before, it only gives an estimation of the potential of the dye in terms of sensitivity. Then, because of the configuration of our experimental system for fluorescence measurement, the excitation light is much more visible on our spectra than for other work in the literature where it is very hard to detect it. As a consequence, regarding the evaluation of the sensitivity we need to suppress from our calculation a rather large spectral band as observed in Fig. 5 where the temperature

dependence might be at its peak. This may impact the final result in comparison to values from the literature. In practice, it might explain our discrepancies with Chaze et al. (2016) regarding the temperature sensitivity of FI-Dis at pH = 5.6 outside of concentration consideration. Nonetheless, it is very interesting to note that for FI-27, we obtain the exact same sensitivity than Chaze et al. (2016) and Sutton et al. (2008) despite this possible effect. Indeed, this could be explained by a very similar sensitivity value in the suppressed spectral band than in the rest of the spectrum, thus not really affecting the calculation of S.

In order to go deeper into the spectral analysis, the next section focuses on the selection of spectral bands for thermometry application in the case of Two/Colors-LIF and comparison with the literature.

3.2.2 Beta function and temperature sensitivity over selected spectral bands

In the literature, some intents for practical use of fluorescent dyes lead sometimes to lower sensitivity than expected from previous spectral characterizations. Indeed, choosing adequate spectral bands, Dunand et al. (2012) found relatively low sensitivity for FI-27 ($S = 0.8 \text{ \%} \cdot \text{C}^{-1}$) under $\lambda_{\text{exc}} = 532 \text{ nm}$ in comparison to previous studies reporting the sensitivity over the whole spectrum. We reproduced this experiment and also used different pH values with pH = 5.6, 7.0 and 9.0. We found similar Beta function and similar values of sensitivity between 20 and 80°C for the same ratio of spectral bands as reported in Tab. 2, although, we used a concentration that was 20 times higher. In this case, as we explained in the sensitivity function section, this lower sensitivity is due to relative homogeneous change of intensity with temperature at any wavelength leading to a much lower sensitivity than the one calculated over the whole spectrum. This means that FI-27, in particular and fluorescein species in general, are very sensitive dyes when excited at 532 nm using 1c-LIF, for one phase thermography measurement for example, but needs to be mixed with another dye when applying the 2c-LIF method (Sutton et al. 2008; Chaze et al. 2017). To this regard, not only the spectral analysis is important but also the calculation of the Beta function as described in Eq. (3) or the whole sensitivity curve as a function of the wavelength (Chaze et al. 2016; Stiti et al. 2019) as it will be described later.

In Fig. 8, an example of the function Beta (λ) is given for FI-27 and FI-Dis at 10^{-5} M calculated between 20 and 80°C for pH = 5.6, 7.0 and 9.0. The excitation wavelength of 532 nm is also traced as well as two spectral bands, selected to be the best choice in order to maximize the sensitivity. The first selected spectral band is 500-520 nm for both dyes located at the highest values of Beta and the second spectral band is at 570-590 nm for FI-27 and 560-580 for FI-Dis corresponding to the lowest value of Beta but with sufficient SNR. Beta values below 480 nm and above 600 nm are spread out because the fluorescence signal is too low so that they shouldn't be used. The values of Beta are averaged in each spectral band and the difference of the resulting values gives the value Δ Beta (λ) reported in Tab. 2. A higher value of Δ Beta (λ) will give a higher sensitivity that is also calculated and reported as well in the same column.

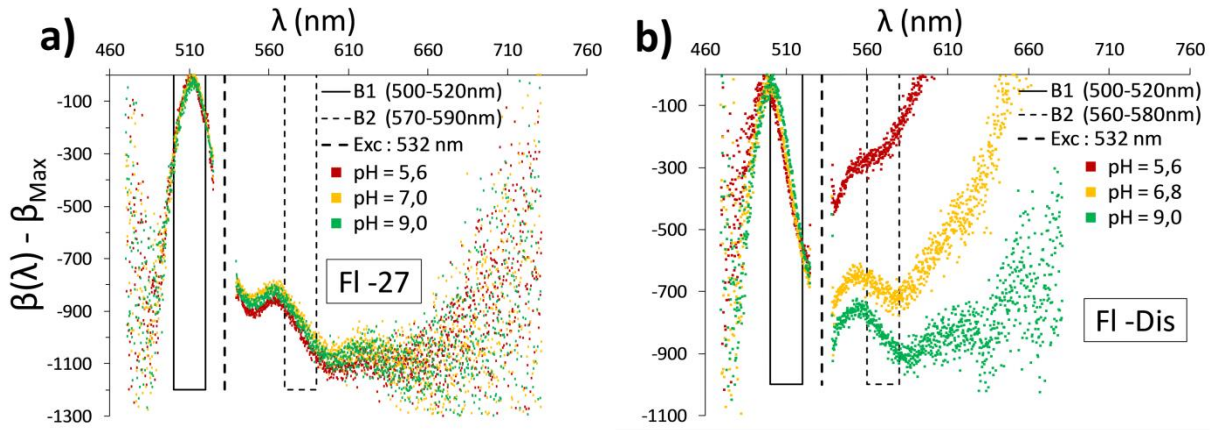


Fig. 8 : Δ Beta as a function of the wavelength for FI-27 in a) and FI-Dis in b) between 20 and 80°C at three different pH.

In Fig. 8 a), the Δ Beta functions are similar for all curves showing a low influence of the pH between 5.6 and 9 on the temperature sensitivity for FI-27. In Fig. 8 b) for FI-Dis, the variation of Δ Beta is narrower but changes a lot above λ_{exc} with the pH. In this case, variations of beta function increase when pH increases between 5.6 and 9. So that at pH = 5.6, the Δ beta function is not changing a lot, meaning that the evolution of fluorescence intensity with temperature is rather homogeneous over the whole spectrum.

In Tab. 2, we report the calculation of Δ Beta function on our experimental results as defined in Eq. (4) for FI-27 and FI-Dis. Concentration, pH and excitation wavelengths are also reported. The two spectral bands were selected in order to maximize the value of Δ Beta.

Dye	Concentration	pH	Excitation (nm)	Spectral bands λ_1, λ_2	Δ Beta (λ) (20 à 80°C) Sensitivity (Eq. (2))
FI-27	5.10 ⁻⁷ M	?	532	$\lambda_1 = 505-515$ nm $\lambda_2 = 570-600$ nm	$\leq 1000 / +0.8 \text{ \%} \cdot \text{°C}^{-1}$ Dunand et al. (2012)
	10 ⁻⁵ M	5.6 9.0	473	$\lambda_1 = 505-525$ nm $\lambda_2 = 540-560$ nm	643.59 / -0.60 %·°C ⁻¹ 666.37 / -0.62 %·°C ⁻¹
		5.6 7.0 9.0	532	$\lambda_1 = 500-520$ nm $\lambda_2 = 570-590$ nm	805.29 / +0.85 %·°C ⁻¹ 752.38 / +0.81 %·°C ⁻¹ 758.85 / +0.82 %·°C ⁻¹
FI-Dis	10 ⁻⁵ M	9.0	473	$\lambda_1 = 515-535$ nm $\lambda_2 = 555-575$ nm	705.62 / -0.66 %·°C ⁻¹
	10 ⁻⁵ M	5.6	532	$\lambda_1 = 500-520$ nm $\lambda_2 = 560-580$ nm	-80 / 0.11 %·°C ⁻¹
		7.0			406 / -0.34 %·°C ⁻¹
		8.0			484 / -0.43 %·°C ⁻¹
		9.0			598 / -0.52 %·°C ⁻¹
10.0	486 / -0.42 %·°C ⁻¹				

Tab. 2 : Δ Beta calculation for FI-27 and FI-Dis for temperature range of 20-80°C with selected spectral bands and sensitivity calculation.

For FI-27, the Δ Beta values reported in Tab. 2 are in the range of 600 to 800 leading to a temperature-sensitivity inferior or equal to +0.85%.C⁻¹. In the case of FI-Dis, the range of Δ Beta values is lower and goes between -80 and 700 leading to temperature sensitivity inferior or equal to -0.66%.C⁻¹.

The results from this table and from previous data on the whole spectrum show that the temperature sensitivity of FI-27 and FI-Dis is very good considering the change of intensity with temperature on the whole spectrum or on a single spectral band (One Color LIF method) but that these dyes are not good candidates taken alone for Two Color LIF-T where they should instead be mixed with another fluorescent dye. This paradoxical characteristic, coming from a rather homogeneous temperature sensitivity over the whole spectral range, is nonetheless very interesting when mixing fluorescein species with another dye. Indeed, it allows a large choice of potential spectral bands and gives versatility when adding another dye as the selection of spectral bands is limited by spectral conflicts or signal intensity.

Another method for analyzing the sensitivity of a dye was introduced by Chaze et al. (2016) and consists in representing directly the curve of sensitivity $S(\lambda)$ instead of Beta as a function of the wavelength. This way, it is easier to select spectral bands presenting the highest sensitivity for One or Two-color/LIF and the same process could be applied to the case of a mixture of dyes (Stiti et al. 2019). In the next section, we will apply this method to our experimental results and will also propose an improvement by calculating each values of $S(\lambda)$ using the sum of intensity over a define spectral range instead of a single wavelength. Furthermore, a discussion is added on the intensity signal as it is evolving strongly over the whole spectrum in order to choose wavelength range in agreement with sufficient SNR. We hope this new method will bring simplification and precision in the process of choosing the best spectral bands for LIF measurement in the future.

3.2.3 Temperature sensitivity for specific wavelength

As proposed by Chaze et al. (2016), we first study the Sensitivity S as a function of the wavelength regarding one of our calibration. In Fig. 9 a), the fluorescence spectra of FI-Dis at pH = 9, $\lambda_{exc} = 532$ nm and $C = 10^{-5}$ M for temperature between -10°C and 80°C with a 10°C step, were normalized by the maximum at 80°C . When the temperature increases in this case, the intensity of fluorescence is increasing over the whole spectrum.

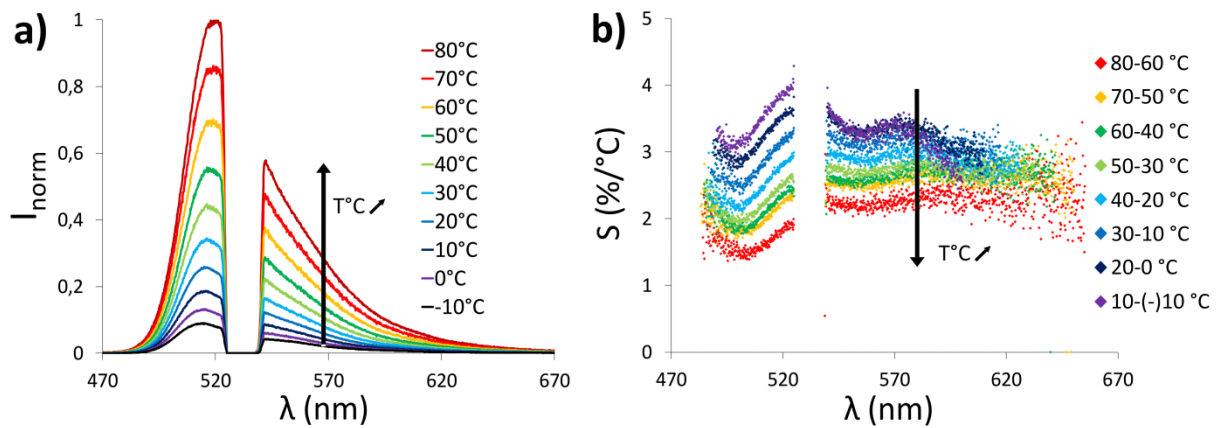


Fig. 9 : Normalized fluorescence intensity in a) and temperature sensitivity in $\% \cdot \text{C}^{-1}$ in b) for FI-Dis at pH = 9 and $C = 10^{-5}$ M.

The corresponding sensitivity $S(\lambda)$, calculated following Eq. (2), is represented in Fig. 9 b) for a temperature range of 20°C and with a step of 10°C . In this case, the sensitivity decreases when the temperature increases. Values of $S(\lambda)$ around -10°C and 80°C , corresponding to the edges of the temperature range are more dispersed as these curves were calculated over a temperature range of only 10°C and were not represented for better visualization. As well, values for each temperature are also dispersed when the intensity signal is low. As a

result, for each curve, the intensity data that are lower than 20% of the maximum of the fluorescence spectrum at -10°C are removed. We can clearly see in b) that the sensitivity is rather continuous for each temperature range. The values are not constant over the whole spectrum but remain of the same order. We can also observe that $S(\lambda)$ has a maximum value below the excitation wavelength for low temperature meanwhile the peak lays above the excitation wavelength at higher temperature which means that the sensitivity decreases more significantly with temperature for $\lambda < 532 \text{ nm}$.

In Fig. 10, we compare different representations of the sensitivity using Eq. (2) for the specific case of FI-27 at 10^{-5} M , at $\text{pH} = 9$, excited at 532 nm and for the temperature range $(-)10 - 20^{\circ}\text{C}$. In blue, the sensitivity is calculated for each wavelength separately (Local S in Fig. 10) as proposed by Chaze et al. (2016). We proposed an improvement of this function with the red curve where each value is calculated using the sum of fluorescence intensity over a spectral band of 20 nm around it. This choice comes from a practical reason as most of the published articles on the topic used a spectral band of this width or very similar. The blue and red curves are roughly giving the same values as a function of the wavelength. The main difference comes from the high oscillation of S for the blue curve which increases when the intensity of fluorescence is lower, meanwhile the red curve shows almost a continuous line from 480 to 730 nm due to the sum of intensity over a relatively large spectral band. When using the red curve to obtain a global sensitivity, the value might be different from the average of the blue curve and the one directly calculated with Eq. (2) over the whole spectrum. This last one is represented in green in Fig. 10. The average value of the red curve gives $S = 2.99 \text{ \%}\cdot\text{C}^{-1}$ against $2.96 \text{ \%}\cdot\text{C}^{-1}$ for the average of the blue curve. Both values are calculated between 500 nm and 600 nm to get rid of the data from the more oscillating zones corresponding to a low fluorescence intensity. The green line corresponds to a sensitivity of $2.86 \text{ \%}\cdot\text{C}^{-1}$ and is mostly independent from the oscillating zones as the value of S is weighted by the intensity value in this case.

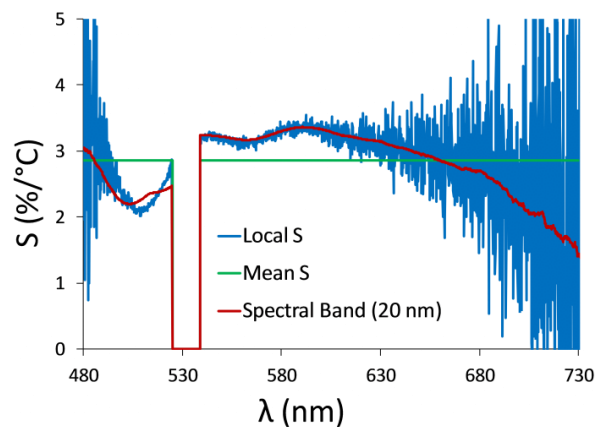


Fig. 10 : Sensitivity functions for FI-27 with $\lambda_{exc} = 532 \text{ nm}$ at $\text{pH} = 5,6$ and for a temperature range of $(-)10 - 20^{\circ}\text{C}$.

Comparing the different curves, we can clearly see the missing information from the value in green as we cannot identify that the sensitivity is higher above 530 nm , going up to $3.35 \text{ \%}\cdot\text{C}^{-1}$, meanwhile it could fell down to $2.1 \text{ \%}\cdot\text{C}^{-1}$ for the blue and red curves below 532 nm instead of $2.86 \text{ \%}\cdot\text{C}^{-1}$ for the green value.

In comparison to the basic function $S(\lambda)$, the red curve follows relatively well the evolution for $\lambda > 530 \text{ nm}$ with much less noise but in the case of $\lambda < 530 \text{ nm}$, it differs significantly. Here, as the blue curve is varying from almost 2 to $3 \text{ \%}\cdot\text{C}^{-1}$ over around 15 nm , the red curve tends instead to averaged the evolution over this typical

spectral range which represents an advantage for spectral band selection when the sensitivity is strongly changing with the wavelength. As a consequence, the principle of the red curve seems the more adequate as it allows to see directly the sensitivity corresponding to a typical spectral band width used in the literature. Furthermore, for Two-Colors LIF-T, we can directly see the resulting sensitivity of using two spectral bands by simply adding the two values that are chosen from the curve in a way that is easier to appreciate than with the basic function $S(\lambda)$ and at the difference of the Beta function. Indeed, the Beta function allows us to see the best combination of spectral bands for obtaining a good ratio of fluorescence for 2c-LIF-T, but the information regarding the value of the resulting sensitivity is lacking and should be calculated aside with original intensity data. For these reasons, we advise future works to use the principle of this modified function $S(\lambda)$ (the red curve in Fig. 10) which could be adapted in term of spectral range.

All our experimental results regarding temperature calibration are sum up in the next two figures for FI-27 and FI-Dis respectively. Our data are represented following our modified function $S(\lambda)$ with values of S calculated over a spectral range of 20 nm. In Fig. 11, the function $S(\lambda)$ is represented for FI-27 at a concentration of 10^{-5} M in different conditions. In a), b) and c), the curves were obtained under the excitation wavelengths of 473 and 532 nm for pH = 5.6, 7.0 and 9.0 respectively. In Fig. 11 d), e) and f), the same curves are gathered by temperature range instead of pH with $T = (-)10-20^{\circ}\text{C}$, $20-50^{\circ}\text{C}$ and $50-80^{\circ}\text{C}$ respectively. Under $\lambda_{\text{exc}} = 473$ nm, the sensitivity is very similar for every experimental condition that we used. The value of $S(\lambda)$ is slightly negative below 530 nm meanwhile it is very close to 0 above this wavelength. Differences appear under $\lambda_{\text{exc}} = 532$ nm where the sensitivity is always positive and shows a maximum for pH = 5,6 with a temperature range of $(-)10-20^{\circ}\text{C}$. The sensitivity is decreasing when increasing the temperature for each pH. Regarding the pH influence, the sensitivity is slightly decreasing when increasing the pH over the whole spectrum for temperature range of $(-)10-20^{\circ}\text{C}$ and $50-80^{\circ}\text{C}$ but not for $20-50^{\circ}\text{C}$ where Fig. 11 e) shows that the sensitivity doesn't change with pH regarding the three values that have been studied.

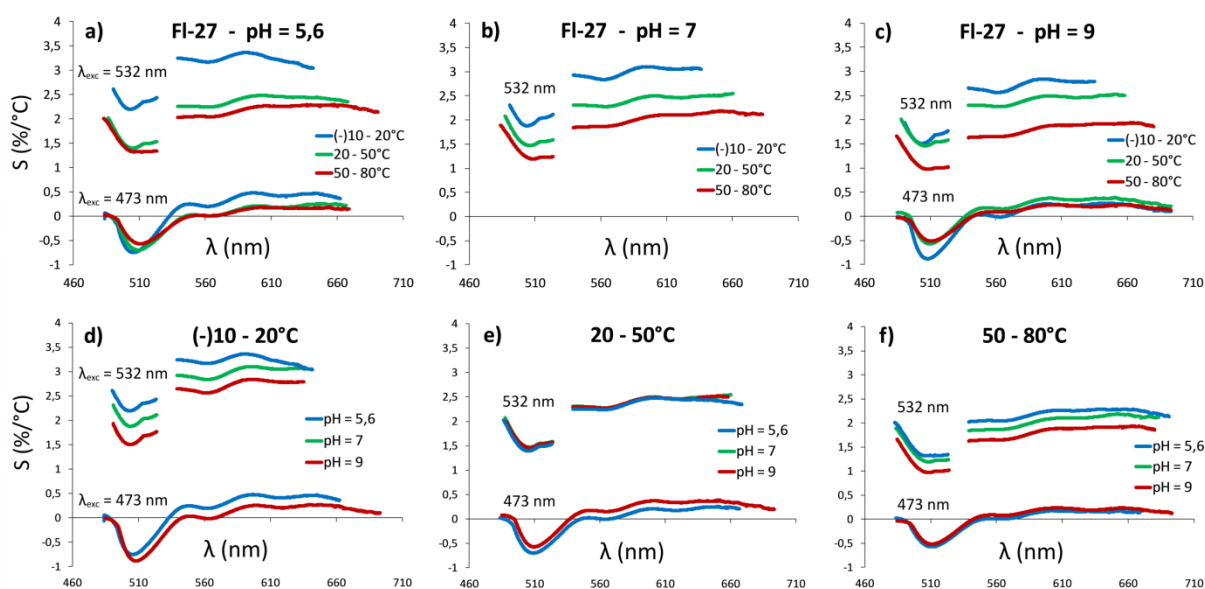


Fig. 11 : Sensitivity function for FI-27 excited at 473 and 532 nm for pH = 5.6, 7.0 and 9.0 in a), b) and c) respectively and by temperature range in d), e) and f).

Another example of the advantage of this method is observed for FI-27 excited at 473 nm. There, the average value of S is close to 0 for every case but we could observe on Fig. 11 d) that S could reach $-0,86 \text{ \%}\cdot\text{C}^{-1}$ at 505 nm for $\text{pH} = 9,0$. When averaged on the whole spectrum, this specific value is hidden among most of the values that are slightly positive (around $0,3 \text{ \%}\cdot\text{C}^{-1}$). Furthermore for $\lambda_{\text{exc}} = 532 \text{ nm}$ in Fig. 11 a), b) and c), the average values of S would give respectively 2.86, 2.55 and 2.24 $\text{\%}\cdot\text{C}^{-1}$ for $\text{pH} = 5,6, 7,0$ and 9,0, meanwhile the curve of the sensitivity is almost constant above 530 nm at all pH and give higher values of around 3,2, 2,9 and 2,7 $\text{\%}\cdot\text{C}^{-1}$ on almost two-thirds of the spectra. It has to be noted that, when calculating the average S , the value is weighted by the intensity of fluorescence which means that the mean of the curve $S(\lambda)$ could give a value that would slightly differ from the ones calculated in Eq. (2) and reported in Tab. 1.

For FI-Dis (Fig. 12), only pH of 9 units was studied under $\lambda_{\text{exc}} = 473 \text{ nm}$ and gives results that are very similar to FI-27 with a slight transition of the curves of 10 nm below. For $\lambda_{\text{exc}} = 532 \text{ nm}$, the temperature sensitivity is always positive as for FI-27. Several differences appear nonetheless. Indeed, the temperature sensitivity is slightly higher for FI-Dis in general. Also, the evolution of $S(\lambda)$ differs above and below 532 nm in comparison to FI-27.

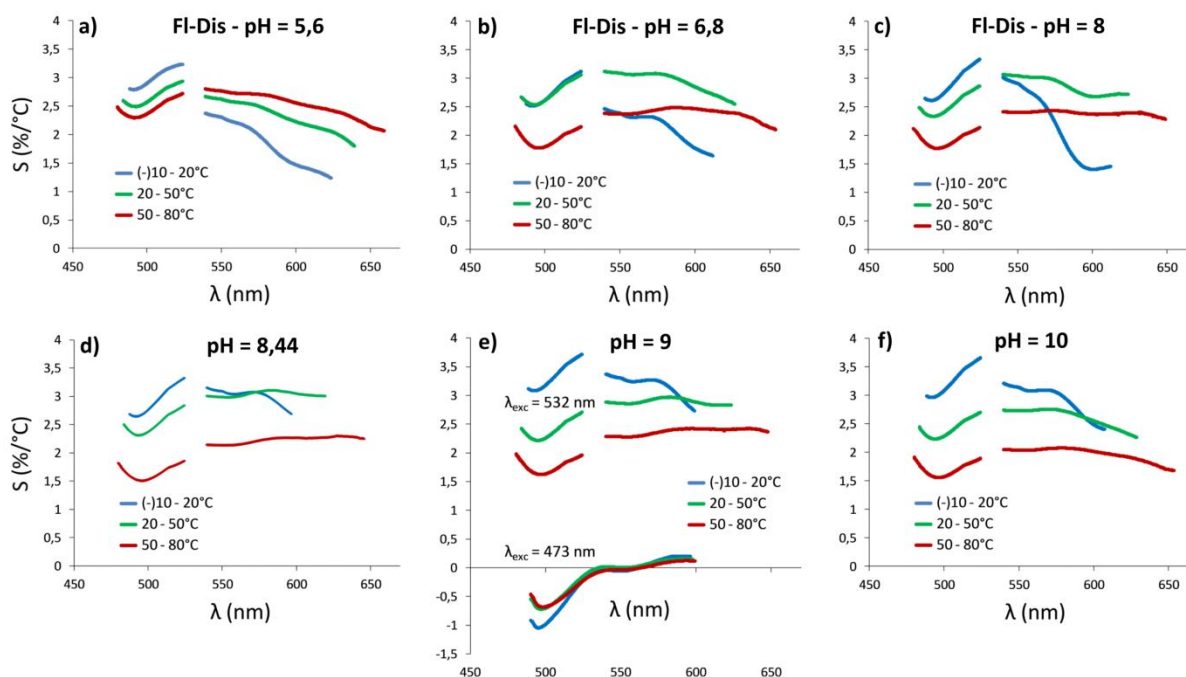


Fig. 12 : Sensitivity function for FI-Dis excited at 473 for $\text{pH} = 9,0$ and 532 nm for $\text{pH} = 5,6, 6,8, 8,0, 8,44, 9,0$ and 10,0 in a), b), c), d), e) and f) respectively.

Now, we will propose two concrete examples of dye and spectral band selections depending on the constraints of experimental measurement. This will allow us to test our method for FI-27 and FI-Dis and to discuss how to manage potential spectral conflicts in the case of One-Dye LIF only. Different types of spectral conflicts can occur for One-dye LIF. First, the absorption spectrum can interfere with the emission spectrum so that the fluorescence is partially absorbed depending on the optical path and the concentration of the dye. When this spectral conflict is not negligible, it is possible to correct the measured signal if the absorption coefficient is not dependent on the temperature and if the concentration of the dye is constant and uniform over the optical path. In the case of Fluorescein species, the absorption coefficient is strongly dependent of the temperature. As a

consequence, it is not possible to correct the signal and this situation should be avoided either by selecting a spectral band of fluorescence away from the absorption spectrum, or by using a small optical path and a low concentration of dye. Regarding another potential spectral conflict, Chaze et al. (2017) pointed out that re-absorption can lead to re-induced fluorescence specially in the case of a high concentration of the dye. They were confronted to this effect as they were using a concentration of $2 \cdot 10^{-4}$ M for FI-Dis. In this case, molecules of dye absorb partially the fluorescence signal over the optical path and emit fluorescence as a consequence. This phenomenon might occur in a successive manner along the optical path and deform the primary fluorescence spectrum as each re-induced fluorescence was produced by an excitation of lower intensity but of various wavelengths that are different from the primary excitation source. This phenomenon should then be avoided as it is very hard if not impossible to correct on the final fluorescence signal. In the case and our two following examples, a concentration of 10^{-5} M allow us to neglect this last effect.

For the first example, we want to measure the temperature in a water tank where the liquid is at ambient temperature and then warmed up thanks to a thermal resistance which is heating up to 45°C . The water is at $\text{pH} = 7$ and original temperature of 25°C . In this case of a single liquid phase, the choice of a single band is sufficient for temperature measurement. Regarding available data from the literature and from this work, the use of FI-27 or FI-Dis under an excitation of 532 nm is the most appropriate as they present the highest temperature sensitivities. The spectral band could then be directly obtained from our curves $S(\lambda)$ established before. As the measurement should occur in a relatively big volume, the absorption effect should be taken into account. From Fig. 13 a), we can clearly see from the $S(\lambda)$ curves that FI-Dis presents higher temperature sensitivity over the whole spectrum at $\text{pH} = 7$ for the temperature range of $20\text{-}50^\circ\text{C}$. Furthermore, we can see that the highest sensitivity is away from the absorption spectrum. We can then select a spectral band between 540 and 580 nm that would have almost identical and very high sensitivity (around $3.0\text{ \%}\cdot^\circ\text{C}^{-1}$) without having the detrimental effect of reabsorption as the coefficient of molar extinction is negligible around these wavelengths.

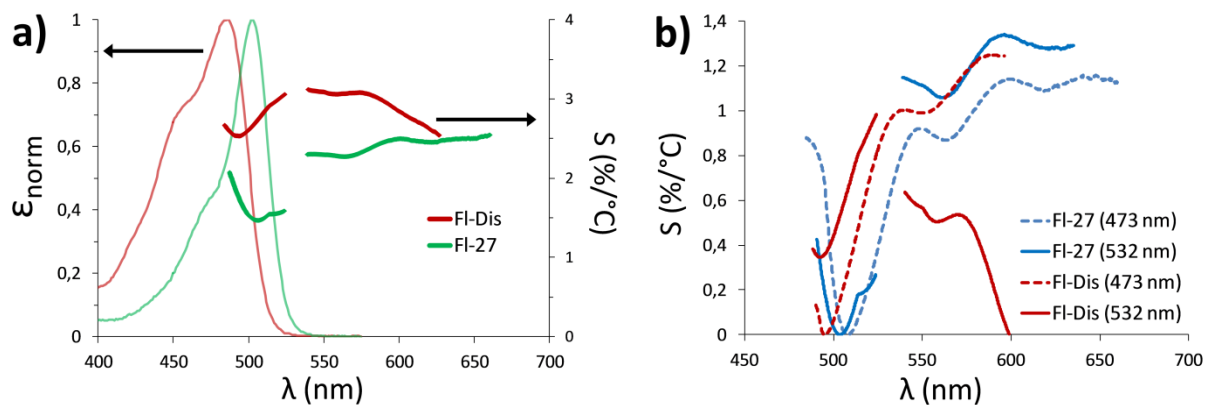


Fig. 13 : Normalized absorption spectra and temperature sensitivity curves for FI-27 and FI-Dis at $\text{pH} = 7$ for $T = 20 - 50^\circ\text{C}$ with $\lambda_{\text{exc}} = 532\text{ nm}$ in a). Temperature sensitivity curves at $\text{pH} = 9$ for $T = (-)10 - 20^\circ\text{C}$ with $\lambda_{\text{exc}} = 473\text{ nm}$ and 532 nm in b).

For the second example, we want to measure temperature change in a cavitating flow with an aqueous solution having a pH of 9 units and circulating through a microchannel similar to what was studied by Ayela et al. (2017). In this case, due to the multiphasic aspect of the problem, 2c-LIF-T should be used to take into account dynamic changes of volume measurement (Dunand et al. 2012). Two spectral bands should then be selected to isolate the temperature in the fluorescence intensity function from other factors. Also, as it is a microscale experiment, the

optical path is small ($\sim 200 \mu\text{m}$) and reabsorption effect can then be neglected. On Fig. 13 b), the $S(\lambda)$ curves were subtracted by their minimum value in order to see which case presents the maximum difference. This allows one to choose two spectral bands for better temperature sensitivity using the 2c-LIF-T method. In this case, even if FI-Dis presents the highest temperature sensitivity in absolute for $\lambda_{\text{exc}} = 532 \text{ nm}$, FI-27 for $\lambda_{\text{exc}} = 532 \text{ nm}$ is most suited here as the maximum difference is the highest and gives a sensitivity of $1,33 \text{ \%}\cdot\text{°C}^{-1}$ by selecting a first band centered at 500 nm and the second at 590 nm . It has to be noted that other dyes could have been used instead of FI-27 or FI-Dis but in order to have an adequate comparison between dyes from the literature, the classic value of sensitivity averaged over the whole spectrum is not sufficient as we discussed previously. Instead, the curve $S(\lambda)$ as proposed by Chaze et al. (2016), or our modified version over a specific spectral band as we did, would be the most appropriate tool to the best of our knowledge.

4 Conclusion

In this work, special emphasis has been given on two Fluorescein species to the purpose of temperature measurement thanks to the use of fluorescent species dissolved in aqueous solution. Although these molecules are relatively well used, a review of their spectral properties under certain critical conditions for LIF technique, like pH or excitation wavelength, from previous literature was needed. Then, we brought new experimental results on the fluorescence of Fluorescein 27 and Fluorescein Disodium by producing temperature calibrations under excitation wavelengths of 473 nm and 532 nm as well as various pH. Very large temperature range from (-10) to 80°C is systematically obtained and might be very useful for future studies on cavitation flow for example, where undercooling might appear under certain experimental conditions. Potential Sensitivity was discussed and compared with data from the literature using similar experimental conditions. Fluorescein species present positive and very high temperature sensitivities when excited at 532 nm . Finally, we emphasize on the necessity to use sensitivity as a function of the wavelength, as advanced by Chaze et al. (2016), or the modified version we proposed in this work for better selection of dye and spectral bands of fluorescence. It represents a clear improvement in comparison to the use of an only averaged value S or Beta function as the impact of fluorescence wavelength is crucial for temperature sensitivity. Two virtual cases were proposed as examples of the use of this method that could also be applied for pH or concentration-sensitivity characterization. In the literature, fluorescein species were also used in combination with other dye in order to increase temperature sensitivity regarding Two-Color LIF measurement. To this regard, the possibility of directly applying our modified curve $S(\lambda)$ to a mixture of two fluorescent species for fast and easy spectral band selection might be studied in future work.

Declarations

Ethical Approval

Not applicable.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Authors' contributions

GL initiated and supervised the work. LP and GL contributed to the study conception and design. Material preparation was performed by LP and GL. Data collection was performed by LP and analysis were performed by LP and GL. The manuscript and all figures were prepared by LP. LP and GL reviewed and approved the manuscript.

Funding

Financial support by the "Agence Nationale de la Recherche" and the "Délégation Générale de l'Armement" (ANR-18-ASTR-0017, project SUPERCAV) is gratefully acknowledged.

Availability of data and materials

Raw data are available from the corresponding author (L. Perrin) upon request.

References

- Ayela F, Cherief W, Colombet D, et al (2017) Hydrodynamic Cavitation through “Labs on a Chip”: From Fundamentals to Applications. *Oil Gas Sci Technol – Rev IFP Energies nouvelles* 72:19. <https://doi.org/10.2516/ogst/2017010>
- Chaze W (2017) Transferts de chaleur et de masse lors de l’impact d’une goutte sur une paroi chaude en régime d’ébullition en film - Application de diagnostics optiques et modélisation. Université de Lorraine
- Chaze W, Caballina O, Castanet G, Lemoine F (2016) The saturation of the fluorescence and its consequences for laser-induced fluorescence thermometry in liquid flows. *Exp Fluids* 57–58
- Chaze W, Caballina O, Castanet G, Lemoine F (2017) Spatially and temporally resolved measurements of the temperature inside droplets impinging on a hot solid surface. *Exp Fluids* 58:96. <https://doi.org/10.1007/s00348-017-2375-1>
- Chen X, Pradhan T, Wang F, et al (2012) Fluorescent chemosensors based on spiroring-opening of xanthenes and related derivatives. *Chem Rev* 112:1910–1956. <https://doi.org/10.1021/cr200201z>
- Coppeta J, Rogers C (1998) Dual emission laser induced fluorescence for direct planar scalar behavior measurements. *Experiments in Fluids* 25:1–15. <https://doi.org/10.1007/s003480050202>
- Demchenko AP (2020) Photobleaching of organic fluorophores: quantitative characterization, mechanisms, protection. *Methods Appl Fluoresc* 8:022001. <https://doi.org/10.1088/2050-6120/ab7365>
- D.L. Silva DL, Coutinho K, S. Canuto S (2010) Electronic spectroscopy of biomolecules in solution - fluorescein dianion in water. *Molecular Physics* 108:3125–3130. <https://doi.org/10.1080/00268976.2010.497779>
- Doughty MJ (2010) pH dependent spectral properties of sodium fluorescein ophthalmic solutions revisited. *Ophthalmic and Physiological Optics* 30:167–174. <https://doi.org/10.1111/j.1475-1313.2009.00703.x>
- Dunand P, Castanet G, Lemoine F (2012) A two-color planar LIF technique to map the temperature of droplets impinging onto a heated wall. *Exp Fluids* 52:843–856. <https://doi.org/10.1007/s00348-011-1131-1>
- Estrada-Pérez CE, Hassan YA, Tan S (2011) Experimental characterization of temperature sensitive dyes for laser induced fluorescence thermometry. *Rev Sci Instrum* 8. <https://doi.org/10.1063/1.3590929>
- Ewinger A, Rinke G, Urban A, Kerschbaum S (2013) In situ measurement of the temperature of water in microchannels using laser Raman spectroscopy. *Chemical Engineering Journal* 223:129–134. <https://doi.org/10.1016/j.cej.2013.01.076>
- Hishida K, Ichianagi M, Kazoe Y, Sato Y (2014) Combined Laser-Based Measurements for Micro- and Nanoscale Transport Phenomena. *Heat Transfer Engineering* 35:125–141. <https://doi.org/10.1080/01457632.2013.812481>

- Ichiyangi M, Sakai K, Kidani S, et al (2012a) Evaluation methodology of gas permeable characterization in a polymer-based microfluidic device by confocal fluorescence imaging. *J Micromech Microeng* 22:065023. <https://doi.org/10.1088/0960-1317/22/6/065023>
- Ichiyangi M, Sasaki S, Sato Y, Hishida K (2009) Micro-PIV/LIF measurements on electrokinetically-driven flow in surface modified microchannels. *J Micromech Microeng* 19:045021. <https://doi.org/10.1088/0960-1317/19/4/045021>
- Ichiyangi M, Sato Y, Hishida K (2007) Optically sliced measurement of velocity and pH distribution in microchannel. *Exp Fluids* 43:425–435. <https://doi.org/10.1007/s00348-007-0326-y>
- Ichiyangi M, Tsutsui I, Kakinuma Y, et al (2012b) Three-dimensional measurement of gas dissolution process in gas–liquid microchannel flow. *International Journal of Heat and Mass Transfer* 55:2872–2878. <https://doi.org/10.1016/j.ijheatmasstransfer.2012.02.009>
- Kim M, Yoda M (2010) Dual-tracer fluorescence thermometry measurements in a heated channel. *Exp Fluids* 49:257–266. <https://doi.org/10.1007/s00348-010-0853-9>
- Lavieille P, Lemoine F, Lavergne G, Lebouché M (2001) Evaporating and combusting droplet temperature measurements using two-color laser-induced fluorescence. *Experiments in Fluids* 31:45–55. <https://doi.org/10.1007/s003480000257>
- Lemoine F, Antoine Y, Wolff M, Lebouche M (1999) Simultaneous temperature and 2D velocity measurements in a turbulent heated jet using combined laser-induced fluorescence and LDA. *Experiments in Fluids* 26:315–323. <https://doi.org/10.1007/s003480050294>
- Leonhardt H, Gordon L, Livingston R (1971) Acid-Base Equilibria of Fluorescein and 2',7'-Dichlorofluorescein in Their Ground and Fluorescent States. *The Journal of Physical Chemistry* 75:245–249. <https://doi.org/10.1021/j100672a011>
- M. Baruah, W. Qin, C. Flors, et al (2006) Solvent and pH Dependent Fluorescent Properties of a Dimethylaminostyryl Borondipyrromethene Dye in Solution. *J Phys Chem A* 110:5998–6009. <https://doi.org/10.1021/jp054878u>
- Okabe K, Inada N, Gota C, et al (2012) Intracellular temperature mapping with a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy. *Nat Commun* 3:705. <https://doi.org/10.1038/ncomms1714>
- Panchompoo J, Aldous L, Baker M, et al (2012) One-step synthesis of fluorescein modified nano-carbon for Pd(ii) detection via fluorescence quenching. *Analyst* 137:2054–2062. <https://doi.org/10.1039/c2an16261j>
- Perrin L, Castanet G, Lemoine F (2015) Characterization of the evaporation of interacting droplets using combined optical techniques. *Exp Fluids* 56:29. <https://doi.org/10.1007/s00348-015-1900-3>
- Podbevsek D (2019) Optical probing of thermodynamic parameters and radical production in cavitating micro-flows. *Université Claude Bernard Lyon 1*
- Ramzay JA (1949) A new method of freezing point determination for small quantities. *J Exp Biol* 26:57–64. <https://doi.org/10.1242/jeb.26.1.57>
- Shafii MB, Lum CL, Koochesfahani MM (2010) In situ LIF temperature measurements in aqueous ammonium chloride solution during uni-directional solidification. *Exp Fluids* 48:651–662. <https://doi.org/10.1007/s00348-009-0758-7>
- Sjöback R, Nygren J, Kubista M (1995) Absorption and fluorescence properties of fluorescein. *Spectrochim Acta, Part A* 51:L7–L21. [https://doi.org/10.1016/0584-8539\(95\)01421-P](https://doi.org/10.1016/0584-8539(95)01421-P)

- Stiti M, Labergue A, Lemoine F, et al (2019) Temperature measurement and state determination of supercooled droplets using laser-induced fluorescence. *Exp Fluids* 60:69. <https://doi.org/10.1007/s00348-018-2672-3>
- Sutton JA, Fisher BT, Fleming JW (2008) A laser-induced fluorescence measurement for aqueous fluid flows with improved temperature sensitivity. *Exp Fluids* 45:869–881. <https://doi.org/10.1007/s00348-008-0506-4>
- Walker DA (1987) A fluorescence technique for measurement of concentration in mixing liquids. *J Phys E: Sci Instrum* 20:217–224. <https://doi.org/10.1088/0022-3735/20/2/019>
- Wang M, Stiti M, Chaynes H, et al (2022) Two-photon fluorescence lifetime imaging applied to the mixing of two non-isothermal sprays: temperature and mixing fraction measurements. *Exp Fluids* 63:172. <https://doi.org/10.1007/s00348-022-03515-5>
- Wu Y, Crua C, Li H, et al (2018) Simultaneous measurement of monocomponent droplet temperature/refractive index, size and evaporation rate with phase rainbow refractometry. *Journal of Quantitative Spectroscopy and Radiative Transfer* 214:146–157. <https://doi.org/10.1016/j.jqsrt.2018.04.034>